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Functional Taxonomy of Bacterial Hyperstructures

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INTRODUCTION

A great deal of effort is currently being put into studying the connections between the constituents of cells as revealed in the transcriptome, proteome, metabolome, and even interactome. Intriguing results have been obtained in terms of scale-free networks and small worlds (107, 219, 276). An indelicate question is on what concept of a cell are such analyses based. It is not certain that biologists understand the essence of even the "simple," well-studied bacterium *Escherichia coli*. Hence, one of the first priorities for students of biological complexity should be to understand what bacterial cells are. A full understanding would entail answering three questions: what are they doing, how are they doing it, and why are they doing it?

Here, we limit ourselves essentially to addressing the second question—how are they doing it—in structural terms, since this is actually easiest insofar as a remarkable degree of structure in bacteria has been revealed over the last decade. This advance in our understanding has led to the proposal that a level of organization exists midway between genes/proteins and whole cells. This is the level of hyperstructures (197). A hyperstructure is more than what is usually meant by a "supramolecular assembly" or a "molecular machine" or even a "module" (2, 96). In our hypothesis, hyperstructures are spatially extended assemblies of molecules and macromolecules that come in nonequilibrium and equilibrium flavors, that command signaling molecules and macromolecules, that interact with one another, and that determine the phenotype of the cell. Hence, in our terminology, the entire eukaryotic nucleolus is a hyperstructure while in that of Hartwell et al., even a single ribosome can be a module (96). Here we summarize the evidence in favor of hyperstructures in bacteria and discuss the possibilities they offer.

PRINCIPLES

The controversy over the importation into biology from physics of concepts such as dissipative structures and Turing structures is far from over. More generally, nonequilibrium structures are proposed to exist that are maintained by a dissipation of free energy, most of the time in the form of a sustained irreversible chemical reaction. Examples of nonequilibrium structures include at the level of the weather, a tornado, and at the level of simple chemicals, the spatiotemporal patterns produced in the Belousov-Zhabotinsky reaction (89). It has also been argued that dissipative structures do not occur in biology but instead that biological structures are close to thermodynamic equilibrium even though there is a flux through them. There is little controversy, though, over the existence of equilibrium structures, such as a fully grown crystal in a saturated solution, that are generally stable for long periods (i.e., their size and composition do not change) in the absence of a source of energy in the environments usually encountered by the bacterium. These structures, which minimize the total free energy of the system, can nevertheless be formed from very mobile constituents. An equilibrium structure may be shifted out of equilibrium if the thermodynamic potentials of its components are also shifted from their equilibrium values. These changes in the values of the chemical potentials may result, for example, from changes in the environment of the cell or from the global nonequilibrium activity of the cell itself. In what follows, we try not to put the cart before the horse. We use these concepts with caution, aware that other, more specifically biological, terms may need to be devised. We shall try to focus first on those extended structures that have been observed to exist or that can reasonably be expected to exist. In doing this, we shall try to class these putative hyperstructures according both to the above concepts and to other, more biologically oriented, concepts.

CANDIDATE HYPERSTRUCTURES

Ribosomal or Nucleolar Hyperstructures?

The nucleolus, a microcompartment within which ribosomes are assembled inside the eukaryotic nucleus, is a fine example of one sort of hyperstructure. It has long been supposed that an equivalent might exist in bacteria where rRNA genes would be transcribed and where ribosomal proteins would assemble onto the nascent rRNA, thus bringing together genes encoding rRNA and ribosomal proteins, nascent RNA, and possibly in the case of bacteria, nascent ribosomal proteins and their genes (284, 290). Evidence that such a nucleolar/ribosomal hyperstructure might exist in *E. coli* has been obtained by fluorescence studies in vivo of RNA polymerase tagged with green fluorescent protein (39); these studies reveal that the distribution of RNA polymerase into transcription foci under conditions of rapid growth corresponds to that expected from RNA polymerase recruitment to a nucleolar hyperstructure to actively transcribe several of the seven sets of rRNA genes present in the chromosome (the authors claim one to three distinct nucleolar hyperstructures per nucleoid [see below]). However, in a search for such a hyperstructure in *Bacillus subtilis* based on the different criteria of localization of Spo0J and *rrn* genes, it was found that only those seven *rrn* genes near the origin are colocalized in transcription foci, while *rrn* genes further away are not colocalized (56); this led to the conclusion that the *rrn* genes may not be in a specific hyperstructure and that their colocalization reflects their position on the chromosome. It is indeed apparent that the two-dimensional position on the chromosome is a very important parameter in the three-dimensional position of the gene within the cell (193, 253, 269) and therefore the hyperstructure. In fact, these results for different organisms can be interpreted as indicating the possibility of more than one nucleolar hyperstructure in a cell as determined in part by the position of genes on the chromosome in terms of strand and neighbors (227). Although a direct demonstration of the existence of the nucleolar hyperstructure and of the nature of its constituents is still needed, it seems reasonable to try to discern certain properties. First, this hyperstructure requires a flow of energy in the form of ATP and GTP hydrolysis. Second, it is observable at high growth rates, when the synthesis of ribosomes consumes most of the bacterium's resources, but not at lower growth rates; this might

mean that it is a dissipative structure which is a nonequilibrium structure that forms only over a certain threshold (we use this narrow definition), although it may simply be a nonequilibrium structure that is smaller and therefore harder to detect at lower growth rates. In other words, this might mean that this hyperstructure is dynamic or metastable, responding to the growth conditions. Third, it involves in its fullest form a variety of macromolecules—DNA, mRNA, rRNA, and proteins—brought together by the spatiotemporal coupling of the processes of transcription, translation, and assembly as far as this is allowed by the position of the genes on the chromosome.

There is also an equilibrium aspect to ribosomes (271). During the stationary growth phase of *E. coli*, 70S ribosomes are converted to translationally inactive, 100S forms of ribosomal dimers by the binding of the ribosome modulation factor. Under energy depletion conditions in a variety of eukaryotes, ribosomes actually form crystal arrays in which they are protected (for references see reference 179); to our knowledge, equivalent hyperstructures of ribosomes in a crystalline state have not been observed in bacteria. As in the case of ribosomes, RNA polymerase is also stored in an inactive but reusable form in the stationary phase: the core enzyme forms complexes with polyphosphate (129), while the sigma70 subunit forms a complex with an anti-sigma factor, RSD (regulator of sigma D) (for references, see reference 271).

A *lac* Hyperstructure as a Paradigm for Transertion Hyperstructures?

It is now believed that the coupling between transcription and translation is the rule rather than the exception. One reason that ribosomes translate nascent mRNA is to prevent the formation of RNA-DNA hybrids (91). Another reason, we believe, is that this coupling generates large hyperstructures. Consider one of the best understood of all genetic systems, the lactose operon in *E. coli*. When the *lac* operon is induced in an exponentially growing culture where it is present in more than one copy, the numbers of transcripts of *lacZ* per cell are 32 full-length, 32 decaying, and 38 nascent transcripts (116). *lacZ* is 3,063 nucleotides long, and under these conditions, the RNA polymerases and ribosomes are 135 and 110 nucleotides apart, respectively. If the short half-life of most mRNA is discounted, in principle, the nascent transcripts are translated by 310 ribosomes. The same operon also contains the *lacY* and *lacA* genes, which can also be cotranscribed and translated. The result should be the formation of a hyperstructure comprising the *lac* genes dynamically attached to tens of nascent mRNAs and to hundreds of ribosomes and the nascent enzymes. Again, this is a nonequilibrium hyperstructure requiring ATP and GTP hydrolysis; again, it is conceivable that a threshold of transcription and translation might have to be exceeded before it takes on a significant identity; again, the structure comprises a variety of macromolecules.

The story does not stop here. LacY is a permease that is inserted into the membrane. If a gene encoding an abundant membrane protein was similarly transcribed, translated, and inserted, there would be hundreds of nascent proteins anchoring the entire hyperstructure to the membrane via "transertion." Transertion is defined as the coupled transcription, translation, and insertion of proteins into and through membranes (27). Transertion in bacteria has been calculated to occupy most of the cytoplasmic membrane and has been established by membrane fractionation and electron microscopy studies (for references, see reference 282) and by supercoiling studies (153). A gene that is transcribed frequently to yield mRNAs that are translated often is, of course, more likely to generate a transertion hyperstructure than a gene that encodes a product that is rare. Given the abundance of complexes of ATP synthase in the cytoplasmic membrane of *E. coli*, it might therefore be expected that transertion would also create a nonequilibrium ATP synthase hyperstructure comprising the *atp* genes, the mRNA, and the nascent proteins. Such transertion hyperstructures might also comprise lipids if the nascent proteins (or the export apparatus that handles them) have preferences for particular phospholipids, which seems to be the case for certain subunits of the ATP synthase (8, 126). It should be noted that the correct folding of the *lac* permease, like that of many other permeases, requires phosphatidylethanolamine (68), and if association with a specific phospholipid can determine the conformation of a protein, reciprocally, the conformation of a protein may determine its association with a phospholipid. Hence, we might expect transertion hyperstructures to play a major role in the structuring of the membrane. This does indeed seem to be the case (27).

The secretory system may also be important in the formation of transertion hyperstructures, although we can see no simple explanation for the different locations of secretory components in different bacteria. In *B. subtilis*, for example, the translocases SecA and SecY are organized in clusters that spiral around the cell (40). This location of SecA is dynamic and depends on both active transcription and translation; it also depends on the presence of anionic phospholipids such as phosphatidylglycerol and cardiolipin. However, SpoIIIJ and YqjG, which are believed to be involved in the transfer of membrane proteins from the Sec apparatus into the membrane, are randomly located in *B. subtilis*, while their homologue in *E. coli* is located mainly at the poles (264). This can be contrasted again with the concentration of Sec translocons at the single export site which is often associated with the division site in the gram-positive coccoid *Streptococcus pyogenes* (232).

There is another sort of multimolecule assembly involving the *lac* operon that is different in both nature and size. In the absence of an inducer such as lactose (or in the presence of the preferred sugar, glucose), the *lac* operon is not transcribed. This is because some of the 10 copies per cell of the tetrameric LacI repressor bind with their dimers to the operator *O1* and to two auxiliary operators, *O2* and *O3*, nearby on the DNA; this on-off binding (which is an equilibrium process) increases the local concentration of LacI at these operators if they are close enough and brings them closer still to increase further the local concentration of LacI at *O1* (188). This structure, albeit small, might be considered a hyperstructure, especially since it serves as a model for much larger ones. In a sense, it is an equilibrium hyperstructure because it is stable in the absence of a flow of energy through that part of the system. We are tempted to term it a "repression" or "site-binding" hyperstructure, and we point out that this equilibrium, site-binding hyperstructure can give way to a nonequilibrium, transertion hyperstructure and vice versa.

Flagellar Hyperstructures

The flagellum is a long helical filament at the surface of a cell, which is responsible for propulsion and is composed of proteins that fall into four classes (154). The basal body transmits the torque from the motor to the hook and filament; it comprises rings in the inner membrane, the periplasm, and the outer membrane; and this part of the flagellum contains fewer than 50 different proteins. The motor/switch system comprises fewer types of proteins and again, these are unlikely to be abundant. The hook, which ensures the orientation of the flagellum to the membrane, also contains relatively few proteins. Finally, the filament itself comprises around 20,000 FliC subunits assembled into a rigid, helical cylinder which contains a channel allowing the export of proteins to the tip. It seems reasonable to suppose that the flagella in *E. coli* are equilibrium structures that do not depend on their activity for their existence (although this is not certain). That said, they do require the proton motive force to function, and they might also be classified as molecular motors (2).

There is, however, another aspect to flagella, and that concerns the possible existence of a transertion hyperstructure. As with the *lac* operon and the *atp* genes, the transertion of the 20,000 or so FliC subunits of the bacterial flagellum might help create a flagellar hyperstructure bringing together the *fliC* gene and neighboring flagellar genes, nascent mRNA, and nascent proteins (38). Flagellar transcripts are organized into three classes, which are synthesized successively. The class 2 genes are under the control of the *flhDC* operon, which encodes transcriptional activators for class 2 promoters; class 2 genes encode the proteins of the hook/basal body and the regulatory proteins FlgM and σ^{28}, and class 3 genes require σ^{28} for their expression (which is inhibited by the anti-σ^{28} FlgM) and encode extracellular proteins such as FliC. A coupling between translation and secretion is achieved via Flk, a membrane-anchored homologue of ribosomal protein S1, which recruits the class 3 mRNA of *flgM* (note that there is also a class 2 mRNA of *flgM* that is not recruited) to allow the translated protein to be exported through the hook/basal body of the flagellum with the aid of FlgN, a specific chaperone (114). The logic is that when the construction of the hook and basal body of the flagellum is sufficiently advanced, FlgM is exported, its inhibition of transcription of the class 3 genes ends, and the synthesis of abundant proteins such as FliC begins. Recently, the flagellar transertion hyperstructure was proposed to act as a sensor of external hydration in *Salmonella enterica* serovar Typhimurium (273). It seems likely that lack of hydration leads to interference of filament subunit polymerization at the growing end of the flagellum, which in turn leads to a backup of nascent subunits of FlgM in the hollow channel, preventing FlgM levels from dropping and transcription of the class 3 genes from occurring. Hence, the growing flagellum itself acts as the sensor. In the model in which hyperstructures determine intracellular events, it should be noted that the feedback signals from the flagellum that switch off the class 3 genes also down-regulate nine virulence genes (273).

Chemosignaling Hyperstructure

In swarmer cells of the differentiating bacterium *Caulobacter crescentus*, the chemotaxis-specific receptors have been localized by immunogold labeling to the polar regions, near the flagellar motor (4, 190). In *E. coli*, these proteins, of which there are five different types (Tar, Tsr, Trg, Tap, and Aer), are also largely at the poles in a hyperstructure that may contain thousands of them (152, 155, 244). So too, it has been shown, are many of the proteins acting downstream in this signal transduction pathway, such as CheA, CheW, and CheR. Protein-protein interactions are one reason for the formation of this hyperstructure (workers in the field use other terms such as array, cluster, and lattice), although protein-lipid affinities cannot be excluded (38). Importantly, the size of the hyperstructure is implicated in the amplification of the signal, an amplification that depends on environmental conditions (35). This is worth stressing: the quantitative sensing of the signal actually depends on there being a hyperstructure. But what sort of hyperstructure is it? This is not easy to answer; it is probably an equilibrium one, since there is no evidence that its size is modulated by a flow of energy (via, for example, ATP or GTP hydrolysis).

Cellulosomal Hyperstructures

Cellulosomes are multienzyme complexes produced by anaerobic cellulolytic bacteria (i.e., not bacteria like *E. coli*) that hydrolyze cellulosic and hemicellulosic substrates of the plant cell wall (15, 60, 67). Cellulosomes consist of a nonenzymatic, fibrillar scaffolding protein, or scaffoldin, which contains binding sites or cohesins for the cellulosomal enzyme subunits (17); these enzymes contain a cohesin-binding site, or dockerin (17), and are positioned periodically along the fibrils (156). Most scaffoldins have between six and nine different cohesins, which can bind up to 26 different cellulosomal enzymes. In fact, cellulosomes can be still more complex than this. In *Acetivibrio cellulolyticus*, there are two primary scaffoldins, ScaA and ScaC, and an adaptor scaffoldin, ScaB, that links ScaA and ScaC. ScaA binds the cellulosomal enzymes and contains a carbohydrate-binding module, type I cohesins, and a type II carboxy-terminal dockerin domain; ScaB binds to the C-terminal dockerin in ScaA through a type II cohesin and binds to a cohesin in the primary anchoring scaffoldin, ScaC, through its C-terminal dockerin. To get an idea of how many enzymes might be bound, consider that ScaA has seven cohesins and a C-terminal dockerin, ScaB has four type II cohesins and a C-terminal dockerin, and ScaC has three cohesins and a C-terminal surface layer homology domain; this has been interpreted as meaning that this complex could bind at least 96 cellulosomal enzymes (67). Cellulosomes are therefore large structures, having masses of 2 to 6 MDa and containing from 14 to 50 polypeptides (60, 131, 164). Indeed, cellulosomes are the largest extracellular "enzyme complexes" known. Large numbers of cellulosomes are found together in hyperstructures or polycellulosomes that can have masses of more than 100 MDa (100) and that form protuberances with diameters of 60 to 200 nm, each containing hundreds of cellulosomes, on the surface of cells (132). Cellulosomes also have other constituents. They contain both carbohydrates and lipids with a high

concentration of unsaturated fatty acids that probably lie between cellulosomes and crystalline cellulose (29, 60), and both cellulosomes and polycellulosomes have the same requirement for reducing agents and calcium (60).

Within the cellulosome, the proteins are organized in a highly ordered chain-like array (164). It is believed that the cellulose-bound cellulosome clusters are the sites of active cellulolysis and that the products are channeled down fibrous structures to the cell (60). Over 95% of the endoglucanase activity of *Clostridium thermocellum* is associated with the cellulosome (25), which is consistent with there being advantages to the enzyme colocalization. This concentration of enzymes acting on related substrates provides the synergy that has been found between cellulases, between cellulases and hemicellulases, and between a cellulosomal enzyme and noncellulosomal enzymes (60, 67). The idea is that the synergy between the enzymes in cellulosomes makes the cellulosome structure more effective in attacking the substrate, as might be the case of the family 9 endoglucanases, which not only cleave cellulose molecules internally but also proceed in a processive manner along the chain from the cleavage site. Moreover, the synergy observed between cellulosomes and noncellulosomal enzymes would also be consistent with direct interactions between them.

Is the cellulosome (or polycellulosome) an equilibrium hyperstructure or a nonequilibrium hyperstructure? Clearly, it can be induced by substrate: cellulosomal genes are expressed as a function of the presence of different substrates, resulting in a population of cellulosomes with activities directed towards the available substrate. Indeed, the growth medium affects both the subunit structure and function of the cellulosome, and when cells are grown on different substrates, such as glucose, cellobiose, xylan, mannan, or pectin, chromatographic fractions of cellulosomes that differ in subunit compositions and enzymatic activities can be obtained (60, 94). In general, protuberances are not produced when the bacteria are grown under cellulase-repressing conditions. Protuberances form in about 4 h when *Clostridium cellulovorans* is grown on cellulose. This does not mean that the hyperstructure is a nonequilibrium one. However, within 5 min of the addition of the soluble sugar glucose, cellobiose or methylglucoside, the protuberances can no longer be detected; in other words, the protuberances dissociate rapidly when no longer needed. Similarly, when *Clostridium thermocellum* is grown on cellobiose, the polycellulosomes are compact and quiescent, while when it is grown on cellulose, the polycellulosomes change morphology radically to form what has been termed "contact corridors" (16). In this case, one interpretation is that the hyperstructure can exist in both equilibrium and nonequilibrium states. It would be interesting to learn whether there is a relationship between the transertion hyperstructure responsible for producing the cellulosomal proteins and the dynamic nature of the polycellulosomal structure.

PTS-Glycolysis Hyperstructures

Proteins involved in metabolic pathways are often reported as existing in the form of multimolecular assemblies, or "metabolons," that allow some form of channeling of intermediates to occur (187, 246, 247, 268). Such metabolons are generally envisaged as containing only a few enzymes that act on successive intermediates in a pathway, which are envisaged as being channeled from one enzyme to the next. One example is the multienzyme complex of tricarboxylic acid cycle enzymes which catalyze the consecutive reactions from fumarate to 2-oxoglutarate in *Pseudomonas aeruginosa* (182).

In the case of glycolysis, there has been controversy over the existence of glycolytic metabolons in prokaryotes and eukaryotes, to which we do not intend to contribute much (48, 168, 211). Rather, we propose to explore the idea of a metabolic hyperstructure in which large numbers of each of the different species of enzymes bind dynamically to one another to increase their local concentration and that of the intermediates. Consider first the phosphoenolpyruvate:sugar phosphotransferase system (PTS), which is responsible for the sensing and uptake of a large number of extracellular sugars and for feeding their products, cytoplasmic sugar phosphates, directly to the enzymes that constitute the glycolytic cycle (233). In *E. coli*, for example, there are many sugar-specific PTS permeases or enzyme II complexes, and each consists of three or four proteins or protein domains, i.e., IIA, IIB, IIC, and sometimes IID. The IIC and IID components are always integral membrane constituents, while the IIA and IIB components are localized to the cytoplasmic surface of the membrane. Glucose transport, for example, depends on a membrane-bound IICBGlc which interacts with a cytoplasmic IIAGlc, and IIAGlc-P is in turn phosphorylated by another cytoplasmic protein, P-HPr. P-HPr derives its phosphoryl group from phosphoenolpyruvate in a reaction catalyzed by enzyme I. Fluorescence studies in vivo of the distribution of enzyme I revealed three patterns of distribution, i.e., polar, punctate, and diffuse, depending, for example, on carbon source, cell density, and growth phase (213). This does not, of course, demonstrate a PTS hyperstructure, since the locations of the other enzymes were not determined. Nevertheless, it does show that the distribution can be nonrandom and that it can change. There are other reasons to suspect the existence of a PTS hyperstructure. For example, it has been proven that IIC is dimeric, and it is likely that the enzymes II form multiprotein complexes with the PTS energy-coupling enzymes, enzyme I, and HPr (for references, see reference 233).

Glucose-6-phosphate, released from the enzyme II complex of the PTS, enters the glycolytic pathway. Evidence also exists for an extensive glycolytic metabolon (245). In eukaryotic cells, interactions between sequential pairs of glycolytic enzymes have been demonstrated, with glycolytic enzymes being partitioned reversibly between cytoplasmic and cytomatrix-bound states depending on physiological conditions (for references, see reference 278) or indeed confined to an organelle, the glycosome, in trypanosomes (117). In *E. coli*, the glycolytic pathway has been isolated as an equimolar multienzyme complex in which compartmentation of substrates was demonstrated. One such complex was reported to have a molecular mass of 1.65 MDa, similar to that calculated for an equimolar complex of the enzymes of glycolysis, and had a particle diameter of 30 to 40 nm (90, 187). Finally, the full enzymatic activity of glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase, and enolase (all glycolytic enzymes) results from their homo-oligomeric association, supporting the idea that a single species of enzyme can be activated to oligomerize by

substrate (259); such an association could help nucleate and stabilize a hyperstructure.

The potential advantages of an enzymatic or metabolic hyperstructure include (i) reduction in the size of the pools of intermediates, since enzymes pass substrates to their partners with minimal delay and, even if the substrate were passed loosely from one enzyme to the next in the pathway so that it sometimes escaped, the presence of identical adjacent enzymes would increase the chance of recapture and the resumption of processing; (ii) protection of unstable or scarce intermediates by maintaining them within the hyperstructure away from other cellular factors that might act on them; (iii) avoidance of an "underground" metabolism in which intermediates become the substrates of other enzymes; and (iv) protection of the cell from toxic or very reactive intermediates that can be transformed rapidly and effectively within the hyperstructure. For example, colocalization within a joint hyperstructure of PTS enzymes actively engaged in sugar transport with glycolytic enzymes engaged in sugar metabolism not only would facilitate the processing of substrates but also could provide enzyme I of the PTS with a high local concentration of the phosphoryl donor for sugar uptake, phosphoenolpyruvate, the product of glycolysis.

There is another important aspect to consider, and that is the extent to which enzymatic hyperstructures are assembled in response to the cell's need for them. Certain transient, dynamic multimolecular assemblies form only in an activity-dependent manner (201, 209, 281), due, for example, to an association between enzymes that occurs only when they are engaged in transporting or transforming substrates or transducing a signal. We have proposed to term these assemblies "functioning-dependent structures" (FDSs) (256). In other words, an FDS assembles when functioning and disassembles when no longer functioning and thus is created and maintained by the very fact that it is in the process of accomplishing a task. This is, in its most useful form, a scale-free definition, and an FDS might describe a group of cells or a hyperstructure of molecules, providing that their performing a task is intrinsic to their assembly. This would lead to advantages for the hyperstructure in addition to those described above, and a functioning-dependent PTS-glycolytic hyperstructure might be expected to have its metabolic activity maximized by active, enzyme-promoted association of membrane and cytoplasmic constituents because (i) the multiple interactions involved in hyperstructure formation would help maintain the hyperstructure during fluctuations in substrate supply; (ii) enzyme association due to substrate-induced binding might select the appropriate transporter from a competing population, during, for example, diauxic growth of a bacterium on two substrate sugars; and (iii) the dissipative nature of the structure would imply that when the substrate was completely exhausted, the membrane domain would disperse, and the cytoplasmic structure would dissociate to free the space for other structures. In eukaryotes, numerous examples of FDSs exist, while in *E. coli*, there is the example of the promotion by substrate binding of the assembly of the three components of the protein-mediated transporter responsible for protein secretion (139).

So what does all this mean? There are equilibrium enzymatic hyperstructures such as the cellulosomes and some large complexes, such as those with the tricarboxylic acid cycle enzymes (182), that might be constituents in hyperstructures, but are there really substrate-induced enzymatic hyperstructures in bacteria? Evidence one way or the other is still insufficient, but since the synthesis of many of these abundant enzymes almost necessarily entails the formation of a synthesis hyperstructure, it is conceivable that the high concentration of enzymes newly released from a synthesis hyperstructure might drive their assembly into an adjoining enzymatic hyperstructure (see below).

Cytoskeletal Hyperstructures

Yet, another class of hyperstructures corresponds to the cytoskeletal networks, which, after many years of disbelief, are now known clearly to exist in bacteria. The FtsZ protein has a structural homology to tubulin (149) and, in vitro, forms a wide variety of polymeric structures depending on the presence and concentrations of lipids, divalent ions, and GTP (88). FtsZ has now been shown to be present in *E. coli* as helices (this is in addition to its presence in the "ring" at the division site [see below]) that have a dynamic activity on the scale of seconds along with slower oscillations of a minute or so (255). FtsZ assembly into filaments is mediated by accessory proteins, reminiscent of the way that microtubule-associated proteins control tubulin assembly into microtubules; these accessory proteins include FtsA, ZipA, and ZapA, of which ZipA is considered to best resemble typical microtubule-associated proteins (6). In chloroplasts, which are related to cyanobacteria, FtsZ exists in the form of both a network and a ring at the site of division (222). FtsZ is also one of the earliest proteins to act in cell division in bacteria, and the question of what lies upstream of the localization of FtsZ to the division site is still unsolved (see "Cell Division Hyperstructures" below).

A range of actin-like proteins also exist in bacteria (for references, see reference 143). After many suspicions that there might be a bacterial actin (for references, see reference 199), which were reinforced by sequence analysis revealing many candidate proteins (32), actin-like filaments were discovered in *B. subtilis* (109), and the crystal structure of MreB was subsequently shown to resemble that of actin (266); filaments formed by MreB have a short pitch (0.73 ± 0.12 mm) and assemble around the middle of the cell, while those formed by Mbl have a longer pitch (1.7 ± 0.28 mm) and cross the entire cell (109). The filamentation of MreB is ATP dependent (266), and the rate of extension of the growing end of filaments is similar to that of actin (0.1 μm/s), generating a potential poleward or centerward pushing velocity at 0.24 μm/min for MreB or Mbl, respectively (59). MreB is an important determinant of cell shape in the rod-shaped *E. coli* and *B. subtilis* as well as in the crescent-shaped *C. crescentus*, where it is reported to organize a PBP 2 complex involved in peptidoglycan synthesis and cell elongation into a band-like structure (76); immunoprecipitation data suggest that this complex contains PBP 1a, PBP 2a, PBP 2b, PBP 3a, and possibly other enzymes responsible for peptidoglycan synthesis (which would be consistent with other evidence for the existence of such a complex) (236). It should be noted that MreB does not appear to play such a role in *B. subtilis* (235). One idea is that MreB filaments not only act as a tracking device for the PBP 2-peptidoglycan biosynthesis complex but also are involved in switching peptidoglycan synthesis from an elongation mode to an invagination

mode required for septation; this is because MreB is localized along the length of the cell during elongation but becomes concentrated at midcell early in division. Indeed, in *Rhodobacter sphaeroides*, MreB is localized to midcell in early septation, where it is also believed to be involved in the control of peptidoglycan synthesis (241). A possible dialogue between cytoskeletal hyperstructures was recently revealed. Separate and independent spirals of MreB and MreC occur in *C. crescentus*, with PBP 2 located both on the MreC spiral and at the division site (66, 70); the pattern of peptidoglycan synthesis depended on the existence of both spirals as well as on the level of FtsZ (70).

MreB may, however, have other cytoskeletal roles, since it appears to form part of a kinetochore-like complex that specifically segregates the replication origin region of the *C. crescentus* chromosome (87). However, the claim that the positioning of the replication hyperstructure in *B. subtilis* is partly dependent on MreB (58) has to be set against evidence that segregation does not depend on MreB in this organism (79). Finally, the motility of *Spiroplasma melliferum*, one of the helical *Mollicutes* which lack cell walls, depends on changes in the length and tension of cytoskeletal structures formed from two proteins, one of which is MreB (128).

In *B. subtilis*, another actin homologue, Mbl, is involved in maintenance of the cell wall. A banded pattern is made along the long axis of the cell by labeled vancomycin binding to sites of peptidoglycan assembly, and this pattern depends on Mbl (54). Turnover occurs along the length of the helical Mbl filaments, which have no obvious polarity and which appear to draw on a cytoplasmic pool that contains oligomers; the filaments are very dynamic, and when labeled and photobleached, they have a recovery half-time of about 8 min (41). The helical pitch of the filaments in cells of various sizes and at different growth rates remains relatively constant. Drawing on earlier ideas (167), it has been proposed that as the cell grows, the newly inserted helical strands of peptidoglycan stretch along the long axis of the cell to generate torsional stress in the direction of helix unwinding, and thus a helical rotation in the cell envelope, in the opposite (left-handed) direction to the growth of the Mbl helical filaments (41). This would enable the filaments to scan the inside surface of the cell membrane and so help generate a uniform new layer of wall material; moreover, the changing pitch of the older peptidoglycan strands would combine with the constant pitch of the newly inserted material via the Mbl filaments to generate a wall resistant to shearing (41). Insofar as peptidoglycan synthesis in *B. subtilis* is dependent on the helical structure of Mbl filaments (rather than simply on a collection of Mbl proteins), the Mbl filaments can be considered a hyperstructure. Similarly, if the segregation of the origins of replication in *C. crescentus* is dependent on the cytoskeletal structures formed by MreB (rather than on some aspect of these proteins that does not involve these structures), they might also be considered hyperstructures. Both are examples of how proteins act at the level of a hyperstructure to perform a cellular task and, arguably, of how a cytoskeletal hyperstructure might interact with other hyperstructures.

The translation elongation factor EF-Tu is a GTPase that delivers amino-acylated tRNAs to the ribosome during the elongation step of translation. EF-Tu/GDP is recycled by the guanine nucleotide exchange factor EF-Ts. The functional homologue of EF-Tu, EF-lα, the eukaryotic aminoacyl-tRNA carrier, is also a major actin-bundling protein in eukaryotes. Bacterial EF-Tu has long been suspected of being an actin homologue. It forms filaments in vitro (18, 51, 98), it is more abundant (5 to 10% of soluble protein) than might be expected if its role were restricted to translation, it associates with the membrane, and its overproduction results in the loss of shape of *E. coli*. Remarkably, it has now been shown to form protofilaments and networks in vivo (163). The nature of the controls over its assembly and disassembly within cells is likely to be interesting, since ribosomes/polysomes were seen to be attached to protofilaments of EF-Tu; in terms of nonequilibrium structures, polymerized EF-Tu exchanges nucleotide rapidly and interacts with the other elongation factor, EF-Ts (18). However, both EF-Tu/GTP and EF-Tu/GDP can polymerize equally well in vitro (see below) (98). Below, we consider the significance of this in terms of ion condensation stabilizing inactive filaments and translational activity destabilizing them. In other words, we consider whether an EF-Tu cytoskeleton might be interpreted as a hyperstructure with a role in the sensing of metabolic activity.

DNA Repair Hyperstructures

In bacteria, DNA damage leads to the induction of the SOS response and the resulting production of more than 40 proteins that mediate diverse DNA repair processes, including nucleotide excision repair (NER) and homologous recombination repair (125, 130, 234). Two proteins play key roles in the regulation of the SOS response: the repressor, LexA, and an inducer comprising the activated, single-stranded-DNA-bound RecA. Several recent studies indicate that SOS-related DNA repair processes involve an orchestrated recruitment of various proteins, leading to the formation of elaborate repair centers also known as repairosomes or repair hyperstructures.

The first SOS proteins to be produced are UvrA, UvrB, and UvrD; these, along with the endonuclease UvrC, catalyze the NER pathway (for a review, see reference 171). In *E. coli*, NER was shown to entail a significant increase of DNA-membrane contact points. To these points, many proteins, including UvrA, UvrC, the four units of RNA polymerase, DNA gyrase, and RecA, are recruited (145). DNA lesions are specifically relocated towards this DNA-protein-membrane hyperstructure, the formation of which depends upon UvrA and RecA. It was proposed that NER, recombination repair, transcription, and replication may have to cooperate with each other (145, 226). The ordered yet fluid nature of the cell membrane is likely to provide a suitable matrix on which these processes can be finely coordinated in space and time.

As a second line of defense, the homologous recombination pathway is activated to repair double-stranded DNA breaks (DSBs) (171). Homologous recombination proceeds through several sequential phases (125). Initially, a presynaptic filament is formed in which RecA molecules coat a single-stranded DNA substrate. This filament then participates in a sequence-specific search for double-stranded DNA sites that are homologous to the RecA-coated segment; a joint species results in which DNA strand exchange and heteroduplex extension occur. It turns out that this elaborate repair pathway involves a large, ordered hyperstructure(s) composed of DNA, many pro-

teins, and presumably the cell membrane (179). How does this repair hyperstructure mature and how does it function?

In *E. coli*, formation of the presynaptic filament depends on the activity of multiple proteins, including the RecBCD, RecN, RecF, RecO, and RecR proteins (31, 125). RecN is an ATPase, a single-stranded-DNA-binding protein, and a member of the structural maintenance of chromosomes (SMC) superfamily (like the eukaryotic Rad50 protein) (119). Following generation of DSBs in *B. subtilis*, RecN assembles at the site of the DSB, followed successively by RecO (believed to be equivalent to Rad52) and RecF, which facilitate the loading of RecA (the equivalent of eukaryotic Rad51) onto single-stranded DNA, while RecF is thought to limit the extent of RecA binding (31, 120, 186). Once formed, RecA nucleofilaments perform a whole-genome search for the homologous duplex. High-resolution electron microscopy studies of *E. coli* cells exposed to DNA-damaging agents indicated that this homologous search is associated with a dramatic reorganization of bacterial chromatin into a tightly packed filamentous structure that appears to be associated with the cellular membrane (141). It has been argued that the tight, striated morphology of the assembly promotes homologous search by attenuating both the sampling volume and the dimensionality of the process (178, 179). Notably, DNA-RecA-membrane association has been proposed to be related to the activation of RecA (85) and to promote the coordination of repair processes (145). A RecA-dependent chromatin reorganization into a highly compacted structure has subsequently been observed to occur in *B. subtilis* cells exposed to DNA-damaging agents (242), implying that the formation of repair hyperstructures is essential and widespread in bacteria (228). The highly dynamic nature of the multiprotein-DNA repairosome or repair hyperstructure(s) was recently highlighted by observations which indicated that the nucleoprotein filaments extend and shrink on a time scale of a minute, a process interpreted as representing the search for the homologous duplex by the nucleofilaments (118). The notion that the repairosome indeed corresponds to a highly regulated hyperstructure is further buttressed by the recent finding that the SOS network exhibits precise temporal modulations (80), indicating the presence of an elaborate signaling network.

Repair of multiple double-stranded DNA breaks also appears to occur in eukaryotic cells in a large DSB repair center or hyperstructure, in which the enzymes in the Mre11-Rad50-Xrs2 complex come to the break sites to control end processing and signaling, followed by proteins that bind to single-stranded DNA as well as other signaling and repair proteins such as Rad51 and Rad52 (147, 148). As in eukaryotes, repair hyperstructures in bacteria are believed to be capable of containing several DSBs, (9), since increasing their number (up to the five or so that a bacterium can handle) does not lead to comparable increases in the number of hyperstructures per cell (120). Correspondingly, the size of this highly dynamic hyperstructure is dependent on the extent of the initial or ongoing DNA damage, and when the SOS response ends, the hyperstructure disappears.

The vectorial search performed by the repair hyperstructure depends on the continuous consumption and dissipation of large amounts of energy. Again, we conclude that it is a nonequilibrium hyperstructure. If exposure to UV irradiation or to other DNA-damaging agents continues such that the rate of damage exceeds that of repair, ATP levels fall, and the dynamic RecA nonequilibrium hyperstructure collapses into a highly ordered RecA-DNA cocrystal where the tight crystalline packaging is believed to protect the DNA by physically sequestering (141). RecA is indeed known to protect chromosomal DNA from degradation. It is worth pointing out that these hyperstructures can be converted into one another depending on conditions (179).

Competence Hyperstructures

Bacteria such as *B. subtilis* are naturally competent and possess numerous proteins that mediate transformation, the binding of DNA to the cell surface, and the transport of this DNA into the cytoplasm, where it can recombine with chromosomal or plasmid DNA. Competence is induced through secreted peptide factors that eventually result in the synthesis of the ComK master transcription regulator of competence. Significantly, in view of phenotypic diversity (see below), competent populations of *B. subtilis* are heterogeneous, and only about 20% of the cells produce ComK. The uptake of DNA requires several groups of proteins (for references, see references 93 and 119). One group includes ComGC, ComGD, ComGE, and ComGG, which are required for the assembly of ComGC into a pseudopilus. This multiprotein structure is believed to channel the double-stranded DNA to a membrane protein, ComEA. The second group of proteins participate in the transport of DNA across the cell membrane and include ComFA, a helicase-like protein associated with the inner face of the membrane. The third group consists of two cytoplasmic proteins, one of which, YwpH, is probably a single-stranded-DNA-binding protein. ComGA is also involved in the assembly of the pseudopilus but has an additional role in the repression of growth and cell division that occurs during competence. After binding, the DNA is cleaved and converted to the single-stranded DNA that is taken up into the cytoplasm through the ComEC membrane permease (only one strand is taken up; the other is degraded). Uptake of DNA is believed to be driven by the ComFA ATPase. We note here that uptake of single-stranded DNA may entail its passage through a pore of poly-β-hydroxybutyrate and polyphosphate (223).

Representatives of each of the above classes of competence proteins (ComGA, ComFA, and YwpH) accumulate preferentially at one (but sometimes both) of the cell poles, as does ComEA, although less markedly (93). In addition to the polar locations, these proteins are also present in a small number of foci close to the membrane, where they appear to follow a helical path. Moreover, given the estimated number of ComEC uptake pores of around 200, these foci are believed to contain many uptake assemblies (93). The interpretation of these results is that the binding, processing, and internalization of foreign DNA occur via molecular machines located at a very few sites and, in the limit, at one site in a cell pole (93, 119).

The story does not end with the internalization of the single-stranded DNA by a large hyperstructure. This DNA is used for homologous recombination with the chromosome, a process that depends on the binding of the ATPase RecA to single-stranded DNA, and as described above, RecN forms repair centers, to which first RecO and later RecF are recruited, on the nucleoids when DNA double-strand breaks occur (120).

Both RecN and RecA have now been found to be localized at one cell pole, albeit with interesting differences. In competent cells, RecA colocalized with ComGA at one cell pole while RecN oscillated between both poles on a time scale of a minute (119). The essence of these findings is that foreign DNA enters the cell at one pole in a competence hyperstructure that includes RecN (which may protect it) and possibly RecA. RecA then forms long dynamic filaments that extend from the competence hyperstructure far into the cell to scan for homologous sequences on the chromosome to allow recombination to occur.

DNA Replication Hyperstructures

The binding of a score of or more copies of the DnaA protein to sites in the origin of replication of *E. coli*, *oriC*, is one of the earliest steps in the initiation of chromosome replication, the assembly of the "orisome" (137) (for reviews, see references 55 and 123); once DnaA in its ATP or ADP form has bound all R boxes in *oriC*, DnaA-ATP specifically binds to other sequences in *oriC* to trigger the opening of the strands and to allow the helicase, DnaB, and DnaC proteins to join the complex, which leads to further separation of the strands and recruitment of the rest of the enzymatic machinery. This process is facilitated by or dependent on the following: supercoiling, the transcription of two genes in the *oriC* region (*mioC* and *gidA*), sequence-specific binding of the proteins FIS and integration host factor to their recognition sites in *oriC*, and the less specific binding of proteins HU and H-NS. The result is an initiation hyperstructure that contains several different sorts of protein, RNA, and *oriC*. The involvement of the membrane in the initiation of replication has a long history (77), and it is tempting to speculate that the initiation hyperstructure may also contain cardiolipin, given that the conversion of DnaA-ADP into DnaA-ATP in vitro requires acidic phospholipids in the bilayer in a fluid state (28, 42, 144). (It is conceivable that more than one initiation hyperstructure may exist when minichromosomes are present [192].) The origin region of *B. subtilis*, like that of *E. coli*, is enriched in membrane fractions, and in *B. subtilis* it has been proposed that the loading of the helicase (see below) requires a membrane-mediated interaction between DnaB and DnaD that constitutes a spatial means to regulate initiation (229).

This initiation hyperstructure matures into a full-blown replication hyperstructure which comprises a DnaE-DnaE or PolC-DnaE strand polymerization complex along with clamp loader, primase, helicase, and single-stranded binding proteins. Other enzymes associated with replication are probably also present. It seems likely that several leading- and lagging-strand polymerases are needed per replication fork and that additional polymerases are required to aid in recombination and repair DNA, as exemplified by the location of RarA in the replication hyperstructure in *E. coli* (134). Ongoing replication requires feeding the hyperstructure with the four deoxyribonucleotides (dNTPs) at the rate of about 3,000 nucleotides per second, yet despite this high rate, there are sufficient dNTPs for only half a minute of synthesis. It is therefore not surprising that there is evidence in both eukaryotes and prokaryotes for the presence of ribonucleoside diphosphate reductase, which catalyzes the synthesis of the dNTPs, in the hyperstructure (92, 161). Dingman originally proposed that in bidirectional replication, the two replication forks remain together in a relatively static complex while the DNA passes through this complex (65); a quarter of a century later, evidence was found in support of this proposal, and this complex was termed the "replication factory" (136). It now seems that the exact spatial and functional relationships of the two forks to one another are uncertain and that the interactions holding the forks within the factory/hyperstructure are dynamic rather than static (36, 173). This picture of a dynamic hyperstructure dependent on its functioning is consistent with the disruption of the hyperstructure that occurs when replication forks encounter the many barriers that result in the arrest or breakage of the fork before reaching the terminus (as in the case of DNA damage and blocking proteins that force a restart by the reassembly of the replication machinery with the help of recombination proteins) (50, 165), when the supply of nucleotides is limited, and when the thymidylate synthetase gene is mutated (183).

Several mechanisms exist in *E. coli* to ensure that once initiation has occurred it does not immediately recur (137). Newly replicated DNA is transiently hemimethylated (with the old strand methylated and the new strand still to be methylated); SeqA, an oligomeric protein, binds preferentially to hemimethylated GATC sites, of which there are 11 in the minimal origin, to sequester this and other regions (such as the *dnaA* gene) and prevent multiple reinitiations. Hundreds of copies of SeqA, along presumably with the DNA to which it binds, form foci (206). There are 19,130 GATC sequences in the *E. coli* chromosome that are clustered around genes implicated in the replication, repair, and structure of DNA as well as in *oriC*; these genes include *dnaA*, *dnaC*, *dnaE*, *gyrA*, *topA*, *hepA*, *lhr*, *parE*, *mukB*, *recB*, *recD*, and *uvrA*, as well as genes involved in the synthesis of the precursors of DNA, purines and pyrimidines, namely, *nrdA*, *purA*, *purF*, *purL*, *pyrD*, and *pyrI* (for references, see reference 200). This has led to the idea of a sequestration/replication hyperstructure based on SeqA that would comprise not just the enzymes responsible for synthesizing DNA but would comprise these enzymes, the enzymes responsible for synthesizing and supplying DNA precursors, enzymes responsible for repair and recombination, the genes encoding many of these enzymes, and a specific region of the membrane (200). It is not surprising, therefore, that SeqA mutants are also affected in supercoiling and in the segregation of the chromosomes. (It should be noted here that formation of a SeqA-based replication hyperstructure is coupled to growth. In an *E. coli* mutant defective in initiation in which DnaC is inactivated for a while and then reactivated by temperature shifts, the number of SeqA foci equals the number of replication forks [11], presumably because the cell has grown too large during the period of inactivation of DnaC for the forks to come together in replication hyperstructures.)

In the hyperstructure approach to replication, the dynamics of the sequestration hyperstructure define the period for which *oriC* is protected from new initiations. *E. coli* contains more than one copy of its chromosome when growing rapidly, and initiation occurs at several copies of *oriC* simultaneously; under these conditions immunofluorescence studies of SeqA suggest that the replication hyperstructure contains up to 6 forks immediately after initiation and that as the cell grows these divide into smaller structures that go to separate locations

(183). This division of the replication/sequestration hyperstructure probably corresponds to the moment when *oriC* becomes available again for initiation. But what explains it? One possibility is that the continuing production of new methylated and hemimethylated GATC sequences titrates away SeqA and so weakens the giant sequestration hyperstructure; in the case of *E. coli* synchronized for chromosome replication, there is evidence that the SeqA foci change in composition as replication proceeds (288). Another, complementary, possibility is related to the existence of a specific segregation machine that might help pull apart both origins and sequestration hyperstructure.

Segregation Hyperstructures

In many bacteria, a hyperstructure based upon centromere-like sequences located around the origins of replication plays a large part in their dynamic movements soon after their replication (but see reference 14). In stalked cells of *C. crescentus*, initiation occurs at the flagellated pole, followed by one of the daughter origins moving to the opposite pole (269). The use of a compound that acts on the actin-like protein MreB (see above) reveals that MreB binds (possibly via another protein, ParB) to a region that includes *oriC* and that this interaction is essential for the segregation of this region of the chromosome (87). Does an MreB hyperstructure move DNA directly? Again in *C. crescentus*, the extended MreB helix relocalizes into a band-like structure at midcell prior to cell division (a change in its location that depends on FtsZ) (76); this change in structure has been likened to the change in the filaments of the actin-like protein ParM, which disassemble after segregating R1 plasmids (185). This plasmid system, which has become a paradigm for segregation of the chromosomes, entails an ATP-dependent assembly of ParM filaments, with another protein, ParR, binding a specific site on the plasmids to pair them (84, 184). In *B. subtilis*, two regions near the origin are involved in positioning them at the poles during vegetative growth (110). This positioning may involve MreB, as mentioned above, which is reported to affect the positioning of the replication hyperstructure (but again see reference 79), as well as another actin-like protein, Mbl, which also affects its positioning but to a lesser degree (58). Somewhat surprisingly, the Spo0J protein is reported not to bind to these regions. Spo0J binds to eight *parS* sites which are distributed over 800 kb on either side of the origin and which are also believed to fold this region into a polar hyperstructure (146). A role for a Spo0J hyperstructure in chromosome segregation would be consistent with the stability conferred on an otherwise segregation-defective plasmid by the cloning of a single *parS* site into it; however, in view of the relatively low percentage of anucleate cells produced in *spo0J* mutants, this hyperstructure might be important for the maintenance of origin location as much as for segregation itself (146). Soj is a protein that interacts directly or indirectly with Spo0J to confer polar location on the origins; this location is believed to entail interaction with DivIVA, a protein situated at the poles (note that DivIVA also has a role in the Min system [see below]). The sporulation-specific protein RacA also interacts with DivIVA (286). During sporulation in *B. subtilis*, a RacA hyperstructure first compacts each daughter chromosome into a single axial filament via a nonspecific binding and then compacts the origin region and helps anchor it to the cell pole by using 25 binding sites distributed over 612 kb around the origin (20). There are therefore at least two hyperstructures implicated in chromosome segregation in *B. subtilis*. Sporulation allows bacteria to survive hostile environments, and it is perhaps significant that the RacA hyperstructure, which is specific for sporulation, is believed to be energy independent (insofar as RacA lacks the ATPase domain that characterizes proteins of this class) (20), whereas a hyperstructure based on MreB dynamics would depend on a supply of energy. In the case of *E. coli*, the origins move from the center of the cell toward the poles and a 25-bp sequence, *migS*, at 211 kb from *oriC* is involved in this migration, which probably only affects the origin region (73, 287). An interesting connection between hyperstructures is the requirement of the presence in the replication hyperstructure of a ribonucleoside diphosphate reductase with the right conformation for chromosome segregation (224). Functional interactions among proteins from the replication hyperstructure (DnaX, SeqA, and ribonucleoside diphosphate reductase) and decatenation and segregation proteins (topoisomerase IV and FtsK) (111, 224, 277) suggest an interaction between replication and segregation hyperstructures to ensure that completion of replication triggers dimer resolution and chromosome segregation.

The above evidence demonstrates the existence of specific hyperstructures that operate on origin regions to either segregate them or maintain them in polar positions. This is not the end of the story. A two-step model has been proposed for *C. crescentus* in which first *oriC* is rapidly moved poleward via MreB and then the rest of the chromosome moves independently of MreB (87). More generally, a two-level model might be envisaged, in which one or more specific hyperstructures operate on the origins but the entire chromosomes are separated by hyperstructure dynamics. In the latter case, a centrally located replication hyperstructure could push out the newly replicated daughters in opposite directions (136), aided and abetted by transcription (69). These ideas have been taken further, and in the strand-specific segregation model it is the association of each parental strand with a particular set of hyperstructures, and the continued association once replication has occurred, that ensures the separation of the daughter chromosomes (227). This is in line with the correlation between the location of genes on the chromosome and their position along the long axis of the cell (193, 253, 269) and with evidence showing a highly asymmetric pattern of segregation of markers around the terminus (275). Recent evidence suggests a progressive separation of the chromosomes (191), but against this there is also good evidence consistent with sister chromosomes remaining linked for a “significant” time after replication and with separation occurring simultaneously throughout the genome (14). It may prove important in this context to distinguish between slowly growing cells, where segregation of daughter chromosomes appears to be straightforward, and rapidly growing cells, where segregation of daughter and granddaughter chromosomes is more complicated and may require grouping into hyperstructures (K. Skarstad, unpublished data). Finally, proteins that compact the chromosomes after replication are also implicated in segregation (see below).

Compaction Hyperstructure

In *E. coli*, the MukB protein is localized to discrete structures, with reports suggesting that it forms either foci at the [1/4] and [3/4] positions during the cell cycle (206) or larger oblongs in the nucleoid (62). In vitro, MukB is associated with MukE and MukF in a large complex (162). MukB is a member of the SMC superfamily (like RecN [see above]), while MukF is a non-SMC protein or kleisin (74). It is generally believed that the MukB, MukE, and MukF proteins form a "condensin" that compacts DNA, probably in association with DNA gyrase, and that this condensin assists in the separation of sister chromosomes. With such a role in chromosome topology, it is not surprising that the MukB and SeqA foci are related. Indeed, the latter are perturbed in both size and distribution in the *mukB* null mutant (208).

Cell Division Hyperstructures

The first discernible step in bacterial cell division (but see below) is the assembly of the tubulin-like protein FtsZ into a ring-like structure, the Z ring, at the nascent division site (72, 230). Almost simultaneously, stabilizing proteins, including the highly conserved ATPase FtsA, assemble on the ring (133). Although FtsZ assembly is clearly coupled to the cell cycle, the precise trigger responsible for initiating Z-ring formation has yet to be determined. FtsA and other early-localizing proteins are thought to anchor the Z ring to the cytoplasmic membrane and to determine its dynamics and stability (7, 215, 216, 249). Another likely constituent of the hyperstructure is GroEL, which depends on FtsZ for its presence at the division site, where it is abundant (205). Reciprocally, FtsZ ring formation at the division site depends on GroEL. (Although this review does not cover *Archaea*, a GroEL cytoskeleton in *Sulfolobus shibatae* has been described [261].) Like its eukaryotic counterpart, the microtubule, the Z ring is a highly dynamic structure. Experiments involving fluorescence recovery after photobleaching indicate that the turnover time for an FtsZ monomer within the ring is between 10 and 30 s (249). Importantly, depleting *E. coli* cells of both FtsA and the membrane-anchored protein ZipA, which is found only in the members of the gamma subdivision of the *Proteobacteria*, prevents the formation of a stable Z ring (215). After formation of a stable Z ring (approximately one-fifth of a mass doubling time later), the formation of the mature division apparatus, or "divisome," is completed by the arrival of the late-localizing proteins (1, 189). These late arrivals include proteins such as FtsK, which is important for ensuring that the chromosome is not bisected by the division septum (10, 26, 86, 106, 142, 274); FtsI, a transpeptidase important for building the cross wall (160, 272); and the amidase AmiC, which plays a role in coupling constriction of the outer membrane and the peptidoglycan layer to the cytoplasmic membrane in *E. coli* (23). Together the proteins that comprise the divisome ensure that the cytoplasmic membrane, the cell wall, and, for gram-negative organisms, the outer membrane constrict in a concerted manner while simultaneously preventing interference with the process of chromosome segregation. This concerted activity directed towards a common objective, cytokinesis, qualifies the divisome as a hyperstructure.

A successful round of cell division requires the coordinated orchestration of numerous cellular processes, including synthesis of proteins and lipids, peptidoglycan synthesis and hydrolysis, and the transport of these newly synthesized materials to the septal site. It is therefore not surprising that the majority of phospholipid synthases in *B. subtilis* are localized to the septal membranes, which are rich in cardiolipin and phosphatidylethanolamine, in an FtsZ-dependent manner (194). During growth in rod-shaped cells, new peptidoglycan is inserted randomly into the expanding lateral envelope, whereas new cell poles are generated de novo as a consequence of cell division (63). Old cell poles are completely inert with respect to peptidoglycan synthesis. The new cell poles are made during a burst of midcell peptidoglycan-synthesizing activity (279). While the absence of functional FtsZ in rod-shaped cells results in filaments that contain lateral peptidoglycan, defective FtsI or FtsQ, enzymes involved in coordinating cross wall synthesis with septation, results in the formation of filamentous cells that have bands of newly synthesized (polar) peptidoglycan at regular positions separated by one average cell length (63). Based on these observations, it has been suggested that the group of cell division proteins that are assembled into the early form of the hyperstructure initiate septal peptidoglycan synthesis by recruiting factors required for cell wall biosynthesis to midcell (1). While at least one component of the divisome is a transpeptidase (FtsI/PBP 3), many are not directly involved in peptidoglycan synthesis and instead must coordinate their activity with those of the peptidoglycan precursor synthetases MurG and MraY (33, 34, 101, 169) as well as the as yet-unidentified precursor translocase. The early cell division proteins and the proteins involved in peptidoglycan metabolism have a common purpose that cannot be achieved individually. Thus, all components involved in the synthesis of septal peptidoglycan represent a single hyperstructure.

In addition to orchestrating the coordinated invagination of the cell membrane(s) and the cell wall, the divisome must also coordinate DNA replication and nucleoid segregation, which are believed to occur simultaneously in bacteria, where the FtsZ ring assembly and DNA replication termination more or less coincide (61). A number of mechanisms are thus present to ensure that the divisome does not bisect the segregating nucleoid. First, DNA-binding proteins (SlmA in *E. coli* [24] and Noc [for nucleoid occlusion] in *B. subtilis* [285]) inhibit FtsZ assembly over the nucleoid, helping to prevent aberrant FtsZ assembly along the length of the cell until chromosome segregation reveals a nucleoid-free space or a membrane domain at midcell (see below). Should disaster occur, two divisome-associated proteins act to ensure that each daughter cell receives a complete copy of genetic material. In *E. coli*, should the nucleoids be accidentally concatenated, FtsK binds near the terminus region of the chromosome and allows the Xer proteins to decatenate the nucleoids (106) prior to the final closure of the invaginating septum. In *B. subtilis* this role is filled by the DNA translocase SpoIIIE. SpoIIIE, a domain of which shares a significant degree of homology with FtsK, is responsible for pumping DNA that has been trapped in the wrong compartment into the appropriate cell prior to cell separation (21).

While the ultrastructure of the cytokinetic ring has yet to be determined, protein localization, mutant analysis, and bacterial

two-hybrid studies (64, 113) indicate that it is held together by an intricate network of interactions between all of its constituents that ultimately serves to link the founder proteins (e.g., FtsZ and FtsA) with the late arrivals (e.g., FtsI). Some of these interactions are formed sequentially on the ring itself, whereas others take place in the cytoplasm prior to FtsZ localization and these preassembled complexes are then loaded onto the ring as a single unit (37). Since the majority of cell division proteins are not present in sufficient numbers to form a ring structure or to interact with FtsZ in a one-to-one ratio, the divisome is envisioned as a ring with protein subcomplexes at regular distances (189). This structure is not a permanent complex but is likely to be very dynamic like the Z ring itself (see above). Given their functional rather than structural role, we envision that the subcomplexes would consist predominantly of late-localizing proteins such as the transpeptidase FtsI and other factors involved in peptidoglycan synthesis and the redirection of growth from lateral to perpendicular. Again the subassemblies localize in three compartments of the cell (i.e., the cytosol, the cytoplasmic membrane, and the periplasm). Regardless of their location or activity, together the individual constituents of the divisome, like those of other hyperstructures (and like, at a lower level, the ribosome), form an integrated structure whose function is greater than the sum of its parts.

The constituents of the division hyperstructure (or the hyperstructure itself) have more than one function. FtsK acts as a DNA translocase to assist chromosome dimer resolution during division when the developing septum may damage dimeric or intercatenated chromosomes (134). Such dimers are produced by homologous recombination, and their resolution involves Xer site-specific recombination whereby XerC and XerD recombine pairs of *dif* sites, which are 28-bp-long sequences located in the 330-kb terminus region where replication terminates (49). This requires interaction between FtsK and XerCD/*dif*.

The concept of a division hyperstructure that comprises many diverse molecules and that matures may help in grappling with a central problem in division. What lies upstream of FtsZ assembly at the division site? What is really the first step in division? What triggers 30% of the FtsZ to go to midcell (249)? It has been observed that cardiolipin domains occur in vivo at the division sites in *E. coli* (122, 174, 175) and *B. subtilis* (115) (note for those who like to look across the phyla: lipid domains are involved in division in fission yeast [220]). One possibility is that the transcription, translation, and insertion of proteins into the membrane (i.e., transertion) structure the bacterial membrane around the chromosomes (27, 78) such that the segregation of the chromosomes creates a domain enriched in cardiolipin between them (196). This domain might then concentrate not only FtsZ (perhaps even in a monomeric form) but also GTP plus other division proteins such as FtsA and ZipA, promoting effective polymerization in the right place at the right time, as well perhaps as other enzymes such as MurG (265). A cardiolipin-rich domain might also concentrate ions such as calcium, which can promote FtsZ polymerization in vitro (150, 289). There is some evidence that calcium levels are higher near the membrane (83) and that they can vary cyclically (43). Intriguingly, production in *E. coli* of S100B, a human protein that undergoes a calcium-dependent conformational change to bind to tubulin, results in it colocalizing with FtsZ and inhibiting division (75).

Does the division hyperstructure also include the genes encoding the enzymes involved in division? Several of these genes are located and transcribed together in the *dcw* cluster at the 2-min position on the *E. coli* chromosome. The 16 genes in this cluster include many involved in division or peptidoglycan synthesis, such as *ftsZ*, *ftsQ*, and *ftsA*. Polymeric proteins such as FtsZ are abundant, with estimates ranging from 3,000 to 15,000 monomers per cell (151), so it is conceivable that this cluster could be attached dynamically to the developing division hyperstructure for at least part of its existence. It may also contain, transiently, the terminus region or at least certain of the repeated motifs believed to direct the translocation activity of FtsK to the *dif* sites (26, 138).

Preventing FtsZ assembly at aberrant locations is critical to maintaining the fidelity of cell division. The Min proteins are one means by which bacteria prevent nonproductive division events at cell poles (140). In *E. coli*, the Min system consists of three proteins, MinC, MinD, and MinE (57). MinC is believed to inhibit the assembly of Z rings by binding to FtsZ polymers and inducing displacement of FtsA or possibly by preventing FtsA binding to FtsZ polymers (for references, see reference 6). MinC is recruited by MinD, which binds cooperatively to membranes in the presence of ATP and which forms a long, helical polymer that assembles from the pole (238). The MinE protein disassembles this structure, and the dynamics are such that the MinCD polymer is assembled first from one pole and then from the other with a period of 30 to 50 seconds. MinD interacts with lipids (251) and, in particular, with anionic phospholipids in vitro (where it forms oligomers) and in vivo (where its distribution depends on these phospholipids) (176); MinD has also been reported to convert phospholipid vesicles into tubes (102). Given the similarity of MinD to dynamin and the role of the latter in membrane insertion during division in eukaryotes, one speculation is that the MinCD polymer contains a tubule of fresh membrane to be incorporated into the membrane when released by MinE (6). Alternatively, the phospholipid tubes may be important constituents of the FtsZ assembly site and the Min system acts to remove them, thereby preventing aberrant Z-ring formation (204).

It should be noted that the Min system is not completely conserved in all bacterial species and is not therefore a universal system for preventing polar septation. In contrast to *E. coli*, *B. subtilis* does not have a MinE homologue and MinCD does not oscillate but remains at the poles through interactions with the DivIVA protein (95). In *C. crescentus* there are no Min homologues. Instead, MipZ, which interacts directly with both the chromosome partitioning apparatus and FtsZ, prevents aberrant FtsZ assembly at cell poles (254). Finally, while it has been suggested that the Min genes play a role in selecting the medial division site, (221), data indicating that the majority of division events take place at midcell even in *min* mutants, along with evidence that the nucleoid plays an important role in the spatial regulation of division, suggest that the primary function of the Min proteins is to prevent aberrant FtsZ assembly at cell poles (140, 204).

CANDIDATE PROCESSES IN HYPERSTRUCTURE ASSEMBLY, DISASSEMBLY, AND INTERACTIONS

A wide variety of factors and processes could be implicated in the assembly and disassembly of hyperstructures. In this section, we revisit those known to be involved in intracellular organization, we present what may be unfamiliar ones, and we indulge in a few frank speculations.

Supercoiling

Gene expression affects the supercoiling of the chromosome, which, it has been argued, constitutes the medium of exchange to discriminate between supercoiling-insensitive promoters and supercoiling-sensitive ones so as to regulate the proportion of the cell's mass occupied by the transcriptional and translational machinery (for a review of this aspect of growth rate control, see reference 260). Rephrased in the language of hyperstructures, this would mean that supercoiling would allow hyperstructures containing genes with supercoiling-insensitive promoters to communicate with the ribosomal hyperstructure(s), which contains the *rrn* genes with their supercoiling-sensitive promoters. In other words, growth rate control is the result of interaction via supercoiling between hyperstructures rather than between individual genes.

Transcription and Translation

The physical coupling of the dynamic, energy-consuming processes of transcription and translation is probably a factor in the formation of many hyperstructures, including the ribosomal hyperstructure. When this coupling is combined with the insertion of the nascent proteins into and through the membrane to give transertion, it becomes, we argue, a major factor. Transertion does not involve just nucleic acids and proteins. It can also involve lipids (see below).

Chromosome Compaction

The chromosome can collapse into a compacted state, as in repair hyperstructures in the absence of available ATP (179). More generally, the tendency of the chromosome to adopt a compacted state is the backdrop to the competition between transertion hyperstructures and other hyperstructures. For these hyperstructures to exist, they must obtain sufficient numbers of RNA polymerases and other enzymes to oppose compaction (283); indeed, inhibition of transcription in *E. coli* by either the antibiotic rifampin or nutrient starvation leads to expansion of the nucleoid (27, 39).

Local Concentrations

The combination of polymeric DNA-binding proteins and cognate binding sites on DNA can, when these sites are closely or regularly spaced, result in the formation of a hyperstructure. Such is the case for LacI binding to auxiliary operators to form a *lac* repression hyperstructure (188) and, as discussed below, for SeqA binding to clusters of GATC sequences. In *B. subtilis*, RacA binding to its preferred sites, which are clustered in the origin-proximal region of the chromosome, is believed to cause the high degree of compaction of this region, while RacA binding with less specificity in the rest of the chromosome causes this to have a lower degree of compaction (20). Local concentrations can involve molecules other than DNA. For example, binding of nascent ribosomal proteins to rRNA may play a role in the formation of a dynamic ribosomal hyperstructure (39, 284). Local concentrations are probably also important in the organizing activity of membrane domains (see below). Finally, a role for locally high concentrations in hyperstructure assembly does not have to involve sites on nucleic acids. Proteins enriched in aromatic amino acids are believed to be particularly flexible and appropriate for interacting with many targets and might constitute "gluons" that could help bind a hyperstructure together (212). (Note that such proteins often correspond to orphans that have no homologues and that are encoded by bacteriophages, which raises the further speculation as to whether bacteriophages manipulate hyperstructures via gluons.)

Distribution of Sequences on Nucleic Acids

To some extent, "the map of the cell is in the chromosome" (53) in the sense that the positions and orientations of genes and other sequences on the chromosome may help underpin the distribution of hyperstructures within the cell (49, 227, 275). In this context, hyperstructures can be envisaged as competing to acquire particular sites on the DNA, such that, for example, an initiation hyperstructure based on DnaA binding to boxes containing GATC sequences might be out-competed by a sequestration hyperstructure based on SeqA binding to GATC sequences and, in turn, this hyperstructure might lose GATC sites to other hyperstructures as they grow during replication.

Chromosome Replication

In principle, the existence of two chemically identical chromosomes in the same cytoplasm allows intracellular differentiation because there is competition between hyperstructures for access to RNA polymerases and to ribosomes (203). Hence, positive feedback circuits can operate whereby the assembly of a hyperstructure based on one chromosome is at the expense of a similar hyperstructure based on the other chromosome. Factors responsible for linking hyperstructures that serve related functions (e.g., the functions related to unstressed growth in rich media) can lead to one coherent pattern of hyperstructures associated with the one daughter chromosome while another pattern of hyperstructures (e.g., related to survival under stress conditions) is associated with the other daughter chromosome. It is then the task of chromosome segregation and cell division to put these different sets of hyperstructures into separate daughter cells to give each a different phenotype appropriate for growth or survival. This argument is underpinned by evidence from bacterial genomes where genes needed for survival under stress conditions are carried on one strand while those needed for growth are carried on the other (227). An extension of this idea is that variations in the rate of replication could, by duplicating different regions of the chromosome at different times, allow a spontaneous differentiation into two sets of hyperstructures and steer cells through the vast space of phenotypes available to them (V. Norris and L. Jan-

niere, unpublished data); this might, for example, help explain the interactions between replication enzymes and those involved in other functions (195) (see below).

Membrane Domain Formation

Hyperstructures may both benefit from and contribute to the existence of domains of different phospholipid compositions within the membranes of *E. coli* and other bacteria. It is clear that the formation and position of domains are closely related to the presence of the chromosome and to transertion (27, 78). Given the phospholipid requirements for membrane permeases (68) and the secretory apparatus (19, 267), it is therefore conceivable that transertion hyperstructures may compete with one another for phospholipids. However, it is also conceivable that the creation of a domain by hyperstructures (note that a domain could be created by the phospholipids excluded from hyperstructures) could concentrate the ions and small molecules needed to nucleate or fuel other hyperstructures (for example, those involved in the initiation of chromosome replication or in cell division, such as the anionic phospholipid cardiolipin and possibly its cationic partner, calcium). Also, although it is tempting to reason in terms of the chemical composition, it is important to be sensitive to the idea that it may be the local viscosity that is the really important parameter.

Phospholipid Turnover, RNA Degradation, and Proteolysis

In the hyperstructure vision of the cell, lipids, nucleic acids, and proteins are better protected inside hyperstructures than outside them. It has long been known that there are two populations of lipids with respect to turnover within bacteria (13), that mRNA generally has a short half-life (in part due to the need to avoid RNA-DNA hybrids) (91), and that proteins outside complexes may encounter proteases (177), as may be the case for the type 4 pilus proteins BfpC, BfpD, and BfpE (52). All we are saying that is unorthodox here is that the turnover of these macromolecules may contribute to the importance of hyperstructures in determining the phenotype, since it would be only within a hyperstructure that these macromolecules could exert their effect. (An alternative to the above would be if the presence of a protein within a degradative hyperstructure was necessary for its degradation; this may be the case for the regulatory protein CtrA, which is degraded when colocalized with the ClpXP protease in the incipient stalked pole of *C. crescentus* [166].)

Intracellular Streaming

The movements of DNA and RNA during transcription and translation may play a role in intracellular currents, a subject that is important in plants (239) but that has yet to be explored in bacteria. The possibility of such movements needs to be explored in the context of hyperstructures. How might different hyperstructures move and by moving affect other hyperstructures? Is there a coupling between movements in the membrane and those in the cytoplasm?

Ions and Ion Condensation

The bacterial cell is full of ions and in particular the monovalent ions potassium and chloride and divalent ions magnesium and calcium, which are involved in a myriad of processes. The hydrated diameter of potassium is around 0.36 to 0.4 nm and that of sodium 0.5 nm, which gives the latter a twofold-greater volume. This greater hydrated volume is believed to be crucial in causing sodium to be excluded from the structured water within the cell (for references, see reference 217). The concept of exclusion from structured water has been applied to the Hofmeister series, in which the proposed order of relative exclusion for ions common in cells is in order of the size of their hydration shell (i.e., the ions with the highest field intensity attract the largest shell of structured water): $Mg^{2+} > Ca^{2+} > Na^{+} > K^{+} > Cl^{-} > NO^{3-}$. The questions for hyperstructure dynamics are to what extent different hyperstructures attract different ions, whether this affects their formation and functioning, and whether the release of ions from one hyperstructure has an influence on another.

There is another, controversial, aspect to ions in biology that has received insufficient attention. Condensation of counterions onto a linear polymer occurs at a critical value of the charge density of the polymer and resembles a phase transition (159). In such condensation, the counterions are delocalized along the polymers, diffuse along the so-called near regions, and can act to bring polymers together. Such polymers include DNA (158) and those formed by actin and tubulin, which have been shown to condense counterions in vitro (for references, see reference 225). It seems reasonable that ion condensation and decondensation could play an important role in the regulation of, and the regulation by, cytoskeletal and transertion hyperstructures in vivo, since these contain charged, linear polymers; indeed, it is possible that ion condensation could allow an entire filamentous network of hyperstructures to act as a single integrative receptor (225). We note that in *B. subtilis*, for example, raised magnesium levels can rescue a mutant defective in the actin-like protein MreB, the twist of cell wall macrofibers is influenced by the concentration of magnesium, and magnesium is important for rescuing certain mutants affected in peptidoglycan synthesis (for references, see reference 79). In vitro, both magnesium and calcium can stimulate polymerization of FtsZ, while calcium can bridge the heads of cardiolipin phospholipids so as to create domains. In the case of the formation of a division hyperstructure, it is conceivable that condensation by one or the other (or both) of these ions onto FtsZ filaments and onto linear arrangements of cardiolipin in the plane of the membrane could underpin a putative interaction between FtsZ and cardiolipin. Such a mechanism might also underpin interactions between polymers of other division proteins and cardiolipin.

Gel/Sol Transitions

In eukaryotic cells, gel/sol transitions are central to many aspects of behavior (for references, see reference 217). A biological gel is a polymer matrix in which the physical cross-links can be formed by a variety of intermolecular interactions, including coulombic, hydrophobic, dipole-dipole, and van der Waals forces as well as hydrogen bonding. If the polymers are

charged, water can fill the interstices to ratios of water to polymer of around 3,000 to 1. Transitions between gel and sol states entail polymer-water interactions giving way to polymer-polymer interactions and vice versa under the influence of stimuli; an increase in the level of calcium has been proposed as one such stimulus, since this divalent cation could bind to anionic sites on polymers to bring them together in a zipper-like fashion and so exclude water (217). In the context of hyperstructures composed of linear polymers, the question is whether the assembly and disassembly of a hyperstructure may both reflect and create gel/sol transitions.

Tensegrity

The architect Buckminster Fuller used the concept of tensegrity to design buildings that combine robustness with an economy of materials. This concept has inspired an architectural approach to biology in which the integrity of the eukaryotic cell is maintained by elastic filaments that provide a continuous tension throughout the structure and rigid struts that resist compression locally (157). Indeed, a considerable body of evidence that the eukaryotic cytoskeleton behaves as a tensegrity structure is accumulating (105). Is this concept relevant to bacteria? Certainly, bacteria have to resist the force of turgor, and the strong peptidoglycan wall allows gram-positive bacteria to resist pressures of up to 20 atmospheres. However, it now appears that bacteria have what we term cytoskeletal hyperstructures. These too can contribute to the structural integrity of the bacterium. *Mycoplasma* spp. for example, lack a peptidoglycan layer and rely on an internal skeleton (97), while even *E. coli* can survive without its peptidoglycan as an L form under isosmotic conditions providing calcium is at the right level (207). The question here is to what extent the dynamics of cytoskeletal hyperstructures are influenced by the mechanical strains imposed by the environment.

Water Structures

It has been argued that, starting from the layer of water structured by contact with a macromolecule, many layers of structured water can be formed (for references, see reference 217, but see also reference 22). Certainly, some, if not all, of the water within the cell is structured by the molecules and macromolecules that constitute the cell (for references, see reference 45). One of the hypotheses in this controversial area is that of two-state water (47, 180, 181), which is proposed to consist of coexisting microdomains of water molecules of different densities and hydrogen bond strengths. Low-density water has a density of 0.91 g/ml, while high-density water has a density of 1.18 g/ml. The two types of microdomains, with different hydrogen-bonded structures, would also differ in all their physical and chemical properties: melting points, boiling points, and solvent properties. These microdomains are in a rapidly exchanging equilibrium, and water at surfaces can be enriched in either type. The implications for biology are considerable (280). For example, could different hyperstructures with different compositions of ions and phospholipids also have different dynamics of water structure, and would such dynamics influence the assembly and functioning of the hyperstructures (see below)? Another provocative hypothesis is that of the water soliton. A soliton has been described as a self-reinforcing solitary wave caused by a delicate balance between nonlinear and dispersive effects in the medium (one possible example is that of a tidal bore). A water soliton is proposed to result from the release of a "ballistic" H^+ from H_2O-H^+ by enzymatic cleavage of ATP. In this model, the ballistic proton generates a cooperative precession of many electrically polarized water molecule dimers that in their excited state form a soliton that moves in a unidirectional flux (258). Such fluxes could occur, for example, along the filaments within cytoskeletal and transertion hyperstructures. Whatever the validity of these or other hypotheses, water is the most abundant constituent of cells, and we should remain open to new developments (237, 291).

INTERACTIONS BETWEEN HYPERSTRUCTURES

If hyperstructures do indeed constitute an intermediate level of organization within bacterial cells, then new types of interaction or new views of old types of interaction between hyperstructures should become apparent. In the unashamed speculations that follow, we take the position that the concept of hyperstructures offers a framework that has its value largely in the new visions it allows, irrespective of the specific nature of these visions.

Hyperstructures Send and Receive Messages via Their Constituents

In our vision of life at the hyperstructure level, proteins, ions, lipids, and other molecules serve as messenger boys or postmen in the communication between hyperstructures rather than as commanders. It is the chemosignaling hyperstructure that instructs CheY to pass the message to the flagellar hyperstructure. It is the flagellar hyperstructure that senses the wetness of the environment and that passes the message on to regulate virulence genes (which may form part of a virulence hyperstructure). The cytoskeletal hyperstructures may also have roles as sensors. In eukaryotes, the homologue to EF-Tu is eEF1α, a GTP-binding protein that mediates association of the aminoacyl-tRNA with the ribosome during the elongation phase of protein synthesis, while the homologue to EF-Ts is eEF1β, a protein that stimulates the exchange of GDP bound to eEF1α for GTP; eEF1β is also an actin-binding protein, and this combined with other evidence for interactions of the translation machinery and the cytoskeleton, such as the cotranslational assembly of certain proteins on the actin cytoskeleton (81), has led to the proposal that a structural relationship between the cytoskeleton and translation apparatus forms the basis for regulation or signaling processes (82). The tubulin cytoskeleton has also been proposed as being intimately related to metabolic activity. Glyceraldehyde-3-phosphate dehydrogenase can interact with microtubules to bring them together (104, 243, 270); triosephosphate isomerase, hexokinase, phosphofructokinase, pyruvate kinase, and aldolase interact with microtubular proteins and a hyperstructure can exist in which enzyme properties and fluxes are altered (210); and pyruvate kinase hinders microtubule assembly in vitro unless phosphoenolpyruvate is present (124).

How might similar couplings between cytoskeletal and met-

abolic hyperstructures work in bacteria, and what functions might they serve (198)? The EF-Tu hyperstructure has polysomes attached (163), which raises the question of whether it could sense directly the translational activity in the cell. For example, translational activity on an EF-Tu hyperstructure might destabilize it and release EF-Tu as a signal to facilitate or inhibit the assembly of other hyperstructures such as the ribosomal one; alternatively, destabilization of the EF-Tu hyperstructure might lead to the decondensation of divalent ions implicated in signaling such as calcium, and, in view of the relationship between charge density and condensation (225), it may be relevant that EF-Tu is phosphorylated in response to calcium (3). Evidence for a dynamic FtsZ hyperstructure is recent (255), but it is conceivable that an eventual interaction between this hyperstructure and glycolytic enzymes might change its dynamics or the availability of monomeric FtsZ to reflect metabolic activity, although of course it is equally conceivable that a nonequilibrium glycolytic hyperstructure could dissociate in the absence of a carbon flux to release glycolytic enzymes to signal to the FtsZ hyperstructure. In this necessarily sketchy and speculative exploration of a putative sensing by hyperstructures, it may be significant that thioredoxin-associated complexes (which are involved in detoxification) isolated from the inner membrane of *E. coli* contain FtsZ and MreB (127).

Coupling via enzyme messengers probably also exists between replication hyperstructures and metabolic hyperstructures. In the yeast *Saccharomyces cerevisiae*, thousands of genes, including those involved in DNA replication, show periodic expression during an ultradian respiration/reduction cycle, while the S phase occurs during the reduction period of the cycle (121, 263) and its completion strictly depends on glyceraldehyde-3-phosphate dehydrogenase through its role in stimulating the expression of histone genes (292); moreover, a key protein in replication, MCM1, is linked genetically to several glycolytic enzymes (44, 46). In human cells, lactate dehydrogenase, phosphoglycerate kinase, and glyceraldehyde-3-phosphate dehydrogenase can be transported to the nucleus, where they can bind single-stranded DNA and, in vitro, modulate the activity of the replicative DNA polymerases Polα, Polδ, and Polε (218, 231, 240); moreover, phosphoglycerate kinase is a cofactor of Polα (108). In prokaryotes, three lines of evidence are consistent with a direct connection between the replication hyperstructure and central carbon metabolism via either diffusible enzymes communicating between hyperstructures or a joint replication/glycolytic hyperstructure. First, in *E. coli*, the velocity of the replication fork may vary from about 1,000 to 200 nucleotides/s as a function of the energy contained in the nutrients (99, 172). Second, *B. subtilis* enzymes of the replication fork (the primase and the helicase) appear to interact directly with metabolic enzymes such as pyruvate dehydrogenase (195; L. Janniere, unpublished data), an enzyme known to modulate the activity of the primase (248). Third, three *B. subtilis* enzymes known or proposed to act on the lagging-strand template in the replicating fork (the DNA polymerase DnaE, the helicase, and the primase) are functionally connected to the five terminal reactions of glycolysis (L. Janniere, unpublished data). The function of such a connection between replication and metabolism might be to generate appropriate phenotypes by relating the speed of replication to the availability of energy so as to modulate copy number and gene expression.

Finally, in the context where enzymes can associate with replication, cytoskeletal, and transertion hyperstructures, it should be noted that the longstanding problem of the signal responsible for the initiation of chromosome replication might have its solution in a competition between hyperstructures; such competition during growth, abetted by positive feedback, might lead to the survival and growth of just a few winners and the release of the constituents (lipids, ions, and proteins such as DnaA) from the losers to signal that initiation is needed (198).

Synergistic Interactions between Processes Lead to the Assembly and Disassembly of Hyperstructures

The view of the eukaryotic cytoskeleton as a tensegrity structure entails attributing a key role to tension in cytoskeletal filaments (105), and it is therefore important to note that the mechanical stretching of a charged linear polymer decreases the charge density and can lead to decondensation of ions (225, 252). Such condensation and decondensation could act on calcium-dependent protein kinases and phosphatases associated with cytoskeletal polymers to further reinforce assembly or disassembly of the polymer. In gram-negative and -positive bacteria, the peptidoglycan layer is the stress-bearing structure, but this does not preclude an interaction between tensegrity and condensation either within the peptidoglycan layer itself due to the stretching of the fibers (see above) or indeed within the cell, whereby, for example, the association between peptidoglycan-synthesizing enzymes and an MreB or Mbl cytoskeletal hyperstructure (in *E. coli* or *B. subtilis*, respectively) might mean that a greater stress would stretch the actin-like filaments to release divalent ions and so stimulate peptidoglycan synthesis and strengthen the wall to resist the stress.

One of the earliest events in cell division is an alteration in the physicochemical properties of the membrane in the center of the cell (78). This alteration may be associated with the formation of the cardiolipin domain, which constitutes part of the putative division hyperstructure (and which may be related to the DNA replication hyperstructure) (174). Metabolism-driven changes to the FtsZ cytoskeletal hyperstructure might lead to both decondensation and release of divalent ions and FtsZ monomers that could then be attracted to the cardiolipin domain (see above). Such attraction might both depend on and result in the formation of large zones of structured and unstructured water in which reactions would proceed differently. These reactions might include the association of FtsZ monomers into polymers to accomplish division. It is not important here whether this scenario is particularly plausible: the point of the example is to illustrate how processes such as membrane domain formation, ion condensation, polymer dynamics, and water structure can be brought together and interpreted within the framework of hyperstructures. Finally, the familiar processes of transcription, translation, and changes in the conformation of enzymes in the presence of substrate may operate synergistically at the level of hyperstructures such that one sort of hyperstructure gives rise to another. For example, it is conceivable that the formation of a transertion hyperstructure (or indeed one based on just coupled transcription and transla-

tion) leads to the assembly of the newly synthesized enzymes in a contiguous metabolic hyperstructure. One condition for this might be that the enzymes produced should associate into homopolymers, a characteristic that is indeed observed for certain glycolytic enzymes (259), and that this association should extend to the nascent proteins. The result would be a joint hyperstructure in a microcompartment of the bacterium specializing in both the synthesis of the enzymes for a pathway and the functioning of that pathway.

Coupled Oscillations Contribute to the Interactions between Hyperstructures

Oscillations of the cell wall in *S. cerevisiae* have a frequency of around 1 kHz and an amplitude of 3 nm and depend on ongoing metabolism and probably the mechanical activities of many proteins (214). The phenomenon of coupled oscillations, first reported by Huygens in 1657 as resulting in the synchronization of pendulum clocks mounted on the same wall, is of general relevance (250). There are many processes that might be coupled across the same constituents within the cell or, more richly, be coupled among themselves. These include processes familiar to biologists such as the polymerization/depolymerization of polymers but also less familiar ones such as condensation/decondensation, fluid/viscous states in the membrane, and gel/sol states in the cytoplasm. In certain systems, one consequence of the coupling of oscillations is to produce solitons, resulting in the oceans in the generation of giant waves. One question is whether a soliton might be generated in an analogous way by hyperstructure-mediated oscillations, and a second is whether it might play a role in the initiation of the cell cycle or other major events in bacterial physiology (258). As a specific example of coupling, consider the following speculation. Enzymes can undergo specific conformational changes on the order of a millisecond that are important in catalysis (71, 103), and the regular conformational changes of identical, neighboring enzymes as they perform their function might be coupled by movements of water structure between the enzymes so that these enzymes move together in a synchronous, low-energy state. It may be worth considering how, at other frequencies, coupling via water dynamics might lead to a synchronization of ribosomes, which undergo major conformational changes as they translate mRNA and which can be packed very closely. Convergence on a common vibrational mode might even allow enzymes in a hyperstructure created by transertion to harness their collective oscillations to allow an economical functioning. The reduction in the restructuring of water by neighboring enzymes moving in synchrony could play a role in both the clustering of the enzymes into a single hyperstructure and interactions between different hyperstructures. In other words, convergence on a common vibrational mode might lead to enzymes coming together in a single hyperstructure and to hyperstructures coming together within the cell. The importance of such vibrational modes might underlie a wide variety of reports of effects of elastic and electromagnetic radiation on biological systems (for references, see references 202 and 262).

The Assembly and Disassembly of Different Hyperstructures Are Coupled

A bacterial cell such as *E. coli* is a compromise solution between a robustness that maximizes survival and an efficiency that maximizes growth. *E. coli* has to both endure long periods in hell and profit from brief periods in heaven. To survive in hell, it must adopt strategies that do not depend on the supply of energy, while to flourish in heaven, it must be prepared to squander energy (and to grow rapidly rather than efficiently). This compromise solution involves equilibrium hyperstructures, which resist dissociation in the absence of a flux of energy/nutrients, and nonequilibrium hyperstructures, which do require such a flux. The explanation is that (i) to survive difficult times, cells contain equilibrium hyperstructures that allow the resumption of key functions for growth when times improve, and (ii) to grow rapidly and distance competitors, cells contain nonequilibrium hyperstructures that allow transport, transcription, translation, signaling, etc. Equilibrium hyperstructures generate nonequilibrium ones as cells go from a survival to a growth regimen, and, vice versa, nonequilibrium hyperstructures generate equilibrium ones as cells go from a growth to survival regimen. A nice example of this is the repair of double-stranded DNA breaks. It has been proposed that exposure to DNA-damaging agents results in the formation of a fibrillar RecA-DNA repairosome (alias a RecA hyperstructure) in which repair depends on energy-consuming processes such as exonuclease and unwinding activities; if exposure continues such that the rate of damage exceeds that of repair, ATP levels falls and the dynamic RecA nonequilibrium hyperstructure collapses into a RecA-DNA cocrystal or equilibrium hyperstructure in which the DNA is nevertheless protected (for references, see reference 179).

The coupling between repair hyperstructures is described above in terms of the availability of ATP. However, a coupling also exists at the level of entropic relationships between hyperstructures. Considerable changes in entropy and water potential are likely to accompany the formation of a polymer in vivo when, for example, ribonucleotides are incorporated into tRNA, rRNA, and mRNA during transcription. It seems likely that these putative changes in entropy have effects on the assembly and disassembly of other hyperstructures, and, for example, an increase in the activity of ribosomal and transertion hyperstructures might be expected to influence the assembly of cytoskeletal hyperstructures. Similarly, the assembly and disassembly of the different cytoskeletal structures might be expected to have indirect effects via water potential on one another; as one hyperstructure disassembles, it releases its constituents, which include monomers, ions, and lipids, and these constituents may directly or indirectly via water structure promote the assembly of another hyperstructure. It may therefore be relevant that in *B. subtilis* MreB filaments are affected by the nature of the nucleoids and by the other actin-like proteins, Mbl and MreBH (58), while in *C. crescentus* the formation of MreB spirals at midcell depend on FtsZ (although other, complementary reasons can be invoked) (76). A picture emerges from these reflections of the bacterial cell as having a range of hyperstructures with the energy flowing down through them, analogous to a large vortex shedding smaller ones or analogous to the way that the electron transport chain

proceeds in steps to finally pass its electron onto oxygen. In that case, it may even be useful to see the cell in terms of a flow of hyperstructures in which, for example, transertion or ribosomal hyperstructures generate cytoskeletal hyperstructures that in turn generate metabolic hyperstructures. This flow would entail small changes of energy between the different classes of hyperstructure.

This energy-oriented description of the cell is not, however, entirely satisfactory. The nature of the internal environment determines whether a hyperstructure is in an equilibrium state or not, but this environment is very much a function of the set of hyperstructures. In addition, this set can vary even in constant external conditions in order, for example, to generate a very diverse population (12, 30, 112). When a *dam* strain of *E. coli* is transformed by fully methylated minichromosomes, these are replicated once only (170). Presumably, the hemimethylated GATC sites are locked away permanently into a sequestration hyperstructure from which they can never be released because the Dam enzyme that should methylate them is lacking. The implication for a wild-type cell is that a hyperstructure that is stable under one intracellular condition becomes unstable under another; moreover, this stability can be a function of the other hyperstructures, which change the ground state and hence convert an equilibrium hyperstructure into one that is out of equilibrium.

DISCUSSION

Rigorous definitions are essential for scientific progress. That said, loose or flexible definitions can also be useful, as in the case of "gene" and "species," particularly in the initial development of a concept. At this stage of the game, we feel it is important to keep options open and to define hyperstructures loosely. Definitions may be based on what hyperstructures contain, what they do, how they do it, and what other entities they do it with. An operational definition would be that large structures that interact with other large structures should be considered hyperstructures. In other words, hyperstructure is as hyperstructure does. A problem would arise here if interactions at the level of hyperstructures were based on processes rarely invoked by biologists. In fact, the hyperstructure idea creates an interpretive framework in which the potential organizing role of a variety of processes, some extremely speculative, can be evaluated; for this reason, we have briefly opened the door here and elsewhere to concepts such as tensegrity, ion condensation, two-state water, giant dipole oscillations, and solitons (for help with these concepts, see references 45, 202, 225, and 258). But too little is known—or accepted—about their relevance to bacterial physiology for us to use these concepts in defining hyperstructures. Initially, the simplest approach may be one of classification according to a few, generally agreed functions. In *E. coli*, candidate hyperstructures include the following. (i) Synthesis hyperstructures are responsible for synthesizing macromolecules in monomer-to-polymer reactions. They often perform coupled transcription, translation, and sometimes insertion into and through membrane, and examples are the ribosomal hyperstructure and the putative *lac* hyperstructure responsible for producing the LacZ, LacY, and LacA proteins. These hyperstructures are dynamic, occupy much of the cell volume, and consume most of its ATP (there may be another class of hyperstructures responsible for synthesizing macromolecules such as polyamines, poly-β-hydroxybutyrate, polyphosphate, and polysaccharides). (ii) Metabolic or enzymatic hyperstructures are responsible for metabolic pathways (such as the putative PTS-glycolytic hyperstructures) and would be characterized by a flow of small metabolites through the system but to assemble (as opposed to function) would not need an input of energy in the form of ATP/GTP hydrolysis. (iii) Signaling hyperstructures are responsible for signal transduction; these include the chemosignaling hyperstructure, in which there is again a flow, here of phosphate and methyl groups. The temptation to put the flagellar hyperstructures in this class should be resisted, since they are physically separate and are created and function differently. (iv) Site-binding hyperstructures, which result from proteins binding to several sites on the chromosome, repress expression or sequester regions. The binding of the repressor of the *lac* operon to the auxiliary operators would make a very small hyperstructure of this type and could be considered an equilibrium structure, while, arguably, the sequestration hyperstructure based on SeqA binding to hemimethylated GATC sites could be considered a much larger example, as could a hyperstructure based on DnaA binding to its boxes. (v) Cytoskeletal hyperstructures are based on homologues to eukaryotic cytoskeletal proteins. The functions of the EF-Tu and FtsZ hyperstructures are unknown, while that of the MreB hyperstructure is believed to be in peptidoglycan synthesis and shape assessment. In none of these cases is it entirely clear whether ATP or GTP hydrolysis is needed for the existence of the hyperstructure. (vi). Energy-responsive hyperstructures undergo radical changes in morphology and activity in response to changes in energy levels. DNA repair hyperstructures in their nonequilibrium form have a strong requirement for ATP but can turn into an equilibrium form in which the DNA is protected when ATP levels fall. (vii). Cell cycle hyperstructures perform the different operations of the cell cycle, including the initiation and elongation steps of chromosome replication, the sequestration and then segregation of newly replicated origins of replication, the compaction of chromosomes, and cell division. This class therefore contains the DnaA-based initiation hyperstructure, the replication hyperstructure itself (and possibly the sequestration hyperstructure, classed above as a binding-site hyperstructure), a putative segregation hyperstructure based on proteins that assist in origin segregation, the compaction hyperstructure based on MukB, the cell division hyperstructure based on FtsZ, and possibly the Min hyperstructure for division site selection or inactivation. The functioning of these hyperstructures requires ATP or GTP, and several of them undergo maturation; indeed, it is conceivable that the initiation hyperstructure matures into a replication hyperstructure that finally nucleates the division hyperstructure and that each disassembles when its task is done.

There are several dimensions along which hyperstructures might be classed. One is the nonequilibrium/equilibrium axis. It seems clear that transertion hyperstructures are nonequilibrium structures and that RecA/DNA cocrystals are equilibrium ones. It is not so clear that this is a useful description of site-binding hyperstructures such as those based on SeqA or on LacI. For example, the *lac* site-binding (or repression) hyperstructure can disassemble if lactose is added (provided that

glucose is absent) to the medium, but it can also disassemble if the nonmetabolizable analogue IPTG (isopropyl-β-D-thiogalactopyranoside) is added. Moreover, some hyperstructures possess both equilibrium and nonequilibrium parts, as is the case for the flagellar hyperstructure at the stage in its development when it can sense the wetness of the environment. A second axis is that of mobile versus static. Transertion and ribosomal hyperstructures owe their existence to the mobility of their constituents while the chemosignaling hyperstructure presumably does not although its operation entails transfer of phosphate groups, and the putative PTS-glycolytic hyperstructures may or may not exist because of changes to the on-off association of their constituents as mediated by metabolites (201); site-binding hyperstructures might be regarded as relatively static although the production of an elevated local concentration requires an on-off binding, and RecA/DNA cocrystals are presumably static. A third axis that might be useful is that of functioning dependence, whereby the functioning of a structure is simultaneously responsible for its formation (256). A PTS hyperstructure for glucose might come together as it starts to import glucose. A *lac* transertion hyperstructure, induced to form by the presence of lactose, might just be included in this category, as indeed might the cell cycle hyperstructures. A fourth axis is that of the energy stored in the hyperstructure. The factors to take into account here vary from the energy stored in the individual components of the hyperstructure (such as the individual polysaccharides in a glycogen granule) to the energy stored in the organization of the hyperstructure to be released when the hyperstructure disassembles.

Such classification would be all very well if a bacterium were just a collection of individual hyperstructures, but is there any more to it? Would the existence of an intermediate level of organization bring the bacterium any advantages, bearing in mind that a general characteristic of an intermediate level is that it filters out noise and buffers information from the level below it and has properties that determine the level above it (135)? These properties stem from the interactions between hyperstructures. We have suggested above that the interplay between hyperstructures determines the hyperstructures that can exist (in other words, we replace the paradigm of genes being responsible for the expression of genes by that of hyperstructures being responsible for the formation of hyperstructures). This interplay may occur through mechanisms that range from the instruction of "messenger boy" ions, proteins, and lipids to esoteric alterations in water structure. These reflections bring us to a picture of the cell as a set of hyperstructures that differ with respect to their energy dissipation and activity; the combined functioning of these hyperstructures creates a changing, integrated intracellular environment in which new hyperstructures can form, in which a hyperstructure can be in an equilibrium state at one moment and not at another (i.e., the ground state changes), and in which the polymerization activity of one set of hyperstructures affects the formation or functioning of another set as part of a progressive stepping-down process of energy dissipation.

Finally, for the concept of hyperstructures to be of real value to microbiologists, it should offer, in addition to the taxonomy attempted above, (i) new interpretations of longstanding problems, as may be the case for the strand separation model for chromosome segregation (227); (ii) the possibility of mathematical modeling leading to testable predictions, a modeling that may take the form of multiagent systems (5); and (iii) experimentally verifiable predictions such as the colocalization of different macromolecules engaged in the same process, a prediction that can be tested by techniques such as secondary ion mass spectrometry (257).

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REFERENCES

1. **Aarsman, M. E., A. Piette, C. Fraipont, T. M. Vinkenvleugel, M. Nguyen-Disteche, and T. den Blaauwen.** 2005. Maturation of the *Escherichia coli* divisome occurs in two steps. Mol. Microbiol. **55:**1631–1645.
2. **Alberts, B.** 1998. The cell as a collection of protein machines: preparing the next generation of molecular biologists. Cell **92:**291–294.
3. **Alexander, C., N. Bilgin, C. Lindschau, J. R. Mesters, B. Kraal, R. Hilgenfeld, V. Erdmann, and C. Lippmann.** 1995. Phosphorylation of elongation factor Tu prevents ternary complex formation. J. Biol. Chem. **270:**14541–14547.
4. **Alley, M. R., J. R. Maddock, and L. Shapiro.** 1992. Polar localization of a bacterial chemoreceptor. Genes Dev. **6:**825–836.
5. **Amar, P., G. Bernot, and V. Norris.** 2004. HSIM: a simulation programme to study large assemblies of proteins. J. Biol. Phys. Chem. **4:**79–84.
6. **Amos, L. A., F. van den Ent, and J. Lowe.** 2004. Structural/functional homology between the bacterial and eukaryotic cytoskeletons. Curr. Opin. Cell Biol. **16:**24–31.
7. **Anderson, D. E., F. J. Gueiros-Filho, and H. P. Erickson.** 2004. Assembly dynamics of FtsZ rings in *Bacillus subtilis* and *Escherichia coli* and effects of FtsZ-regulating proteins. J. Bacteriol. **186:**5775–5781.
8. **Arechaga, I., B. Miroux, S. Karrasch, R. Huijbegts, B. de Kruijff, M. J. Runswick, and J. E. Walker.** 2000. Characterisation of new intracellular membranes in *Escherichia coli* accompanying large scale overproduction of the b subunit of F_1F_0 ATP synthase. FEBS Lett. **482:**215–219.
9. **Aten, J. A., J. Stap, P. M. Krawczyk, C. H. C. H. van Oven, R. A. Hoebe, J. Essers, and R. R. Kanaar.** 2004. Dynamics of DNA double-strand breaks revealed by clustering of damaged chromosome domains. Science **303:**92–95.
10. **Aussel, L., F. X. Barre, M. Aroyo, A. Stasiak, A. Z. Stasiak, and D. Sherratt.** 2002. FtsK Is a DNA motor protein that activates chromosome dimer resolution by switching the catalytic state of the XerC and XerD recombinases. Cell **108:**195–205.
11. **Bach, T., M. A. Krekling, and K. Skarstad.** 2003. Excess SeqA prolongs sequestration of *oriC* and delays nucleoid segregation and cell division. EMBO J. **22:**315–323.
12. **Balaban, N. Q., J. Merrin, R. Chait, L. Kowalik, and S. Leibler.** 2004. Bacterial persistence as a phenotypic switch. Science **305:**1622–1625.
13. **Ballesta, J. P. G., C. L. De Garcia, and M. Schaechter.** 1973. Turnover of phosphatidylglycerol in *Escherichia coli*. J. Bacteriol. **116:**210–214.
14. **Bates, D., and N. Kleckner.** 2005. Chromosome and replisome dynamics in *E. coli*: loss of sister cohesion triggers global chromosome movement and mediates chromosome segregation. Cell **121:**899–911.
15. **Bayer, E. A., J. P. Belaich, Y. Shoham, and R. Lamed.** 2004. The cellulosomes: multienzyme machines for degradation of plant cell wall polysaccharides. Annu. Rev. Microbiol. **58:**521–554.
16. **Bayer, E. A., and R. Lamed.** 1986. Ultrastructure of the cell surface cellulosome of *Clostridium thermocellum* and its interaction with cellulose. J. Bacteriol. **167:**828–836.
17. **Bayer, E. A., E. Morag, and R. Lamed.** 1994. The cellulosome-a treasure trove for biotechnology. Trends Biotechnol. **12:**378–386.
18. **Beck, B. D.** 1979. Polymerization of the bacterial elongation factor for protein synthesis, EF-Tu. Eur. J. Biochem. **97:**495–502.
19. **Benach, J., Y. T. Chou, J. J. Fak, A. Itkin, D. D. Nicolae, P. C. Smith, G. Wittrock, D. L. Floyd, C. M. Golsaz, L. M. Gierasch, and J. F. Hunt.** 2003. Phospholipid-induced monomerization and signal-peptide-induced oligomerization of SecA. J. Biol. Chem. **278:**3628–3638.
20. **Ben-Yehuda, S., M. Fujita, X. S. Liu, B. Gorbatyuk, D. Skoko, J. Yan, J. F. Marko, J. S. Liu, P. Eichenberger, D. Z. Rudner, and R. Losick.** 2005. Defining a centromere-like element in *Bacillus subtilis* by identifying the

binding sites for the chromosome-anchoring protein RacA. Mol. Cell **17:** 773–782.

21. **Ben-Yehuda, S., D. Z. Rudner, and R. Losick.** 2003. Assembly of the SpoIIIE DNA translocase depends on chromosome trapping in *Bacillus subtilis*. Curr. Biol. **13:**2196–2200.
22. **Berkowitz, M. L., D. L. Bostick, and S. Pandit.** 2006. Aqueous solutions next to phospholipid membrane surfaces: insights from simulations. Chem. Rev. **106:**1527–1539.
23. **Bernhardt, T. G., and P. A. de Boer.** 2003. The *Escherichia coli* amidase AmiC is a periplasmic septal ring component exported via the twin-arginine transport pathway. Mol. Microbiol. **48:**1171–1182.
24. **Bernhardt, T. G., and P. A. de Boer.** 2005. SlmA, a nucleoid-associated, FtsZ binding protein required for blocking septal ring assembly over chromosomes in *E. coli*. Mol. Cell **18:**555–564.
25. **Bhat, K. M., and T. M. Wood.** 1992. The cellulase of the anaerobic bacterium *Clostridium thermocellum*: isolation, dissociation, and reassociation of the cellulosome. Carbohydr. Res. **227:**293–300.
26. **Bigot, S., O. A. Saleh, C. Lesterlin, C. Pages, M. El Karoui, C. Dennis, M. Grigoriev, J. F. Allemand, F. X. Barre, and F. Cornet.** 2005. KOPS: DNA motifs that control *E. coli* chromosome segregation by orienting the FtsK translocase. EMBO J. **24:**3770–3780.
27. **Binenbaum, Z., A. H. Parola, A. Zaritsky, and I. Fishov.** 1999. Transcription- and translation-dependent changes in membrane dynamics in bacteria: testing the transertion model for domain formation. Mol. Microbiol. **32:**1173–1182.
28. **Boeneman, K., and E. Crooke.** 2005. Chromosomal replication and the cell membrane. Curr. Opin. Microbiol. **8:**143–148.
29. **Bolobova, A. V., A. V. Zhukov, and A. A. Klyosov.** 1994. Lipids and fatty acids in cellulosomes of *Clostridium thermocellum*. Appl. Microbiol. Biotechnol. **42:**128–133.
30. **Booth, I. R.** 2002. Stress and the single cell: intrapopulation diversity is a mechanism to ensure survival upon exposure to stress. Int. J. Food Microbiol. **78:**19–30.
31. **Bork, J. M., M. M. Cox, and R. B. Inman.** 2001. The RecOR proteins modulate RecA protein function at 5′ ends of single-stranded DNA. EMBO J. **20:**7313–7322.
32. **Bork, P., C. Sander, and A. Valencia.** 1992. An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and hsp70 heat shock proteins. Proc. Natl. Acad. Sci. USA **89:**7290–7294.
33. **Bouhss, A., M. Crouvoisier, D. Blanot, and D. Mengin-Lecreulx.** 2004. Purification and characterization of the bacterial MraY translocase catalyzing the first membrane step of peptidoglycan biosynthesis. J. Biol. Chem. **279:**29974–29980.
34. **Boyle, D. S., and W. D. Donachie.** 1998. MraY is an essential gene for cell growth in *Escherichia coli*. J. Bacteriol. **180:**6429–6432.
35. **Bray, D., M. D. Levin, and C. L. Morton-Firth.** 1998. Receptor clustering as a cellular mechanism to control sensitivity. Nature **393:**85–88.
36. **Breier, A. M., H. U. Weier, and N. R. Cozzarelli.** 2005. Independence of replisomes in Escherichia coli chromosomal replication. Proc. Natl. Acad. Sci. USA **102:**3942–3947.
37. **Buddelmeijer, N., and J. Beckwith.** 2004. A complex of the *Escherichia coli* cell division proteins FtsL, FtsB and FtsQ forms independently of its localization to the septal region. Mol. Microbiol. **52:**1315–1327.
38. **Cabin-Flaman, A., C. Ripoll, M. H. J. Saier, and V. Norris.** 2005. Hypothesis: chemotaxis in *Escherichia coli* results from hyperstructure dynamics. J. Mol. Microbiol. Biotechnol. **10:**1–14.
39. **Cabrera, J. E., and D. J. Jin.** 2003. The distribution of RNA polymerase in *Escherichia col*i is dynamic and sensitive to environmental cues. Mol. Microbiol. **50:**1493–1505.
40. **Campo, N., H. Tjalsma, G. Buist, D. Stepniak, M. Meijer, M. Veenhuis, M. Westermann, J. P. Muller, S. Bron, J. Kok, O. P. Kuipers, and J. D. Jongbloed.** 2004. Subcellular sites for bacterial protein export. Mol. Microbiol. **53:**1583–1599.
41. **Carballido-Lopez, R., and J. Errington.** 2003. The bacterial cytoskeleton: in vivo dynamics of the actin-like protein Mbl of *Bacillus subtilis*. Dev. Cell **4:**19–28.
42. **Castuma, C. E., E. Crooke, and A. Kornberg.** 1993. Fluid membranes with acidic domains activate DnaA, the initiator protein of replication in *Escherichia coli*. J. Biol. Chem. **268:**24665–24668.
43. **Chang, C. F., H. Shuman, and A. P. Somlyo.** 1986. Electron probe analysis, X-ray mapping, and electron energy loss spectroscopy of calcium, magnesium, and monovalent ions in log-phase and in dividing *Escherichia coli* B cells. J. Bacteriol. **167:**935–939.
44. **Chang, V. K., J. J. Donato, C. S. Chan, and B. K. Tye.** 2004. Mcm1 promotes replication initiation by binding specific elements at replication origins. Mol. Cell. Biol. **24:**6514–6524.
45. **Chaplin, M.** 2006. Do we underestimate the importance of water in cell biology? Nat. Rev. Mol. Cell Biol. **7:**861–866.
46. **Chen, Y., and B. K. Tye.** 1995. The yeast Mcm1 protein is regulated posttranscriptionally by the flux of glycolysis. Mol. Cell. Biol. **15:**4631–4639.
47. **Cho, H. C., S. Singh, and G. W. Robinson.** 1997. Understanding all of water's anomalies with a non-local potential. J. Chem. Phys. **107:**7979–7988.
48. **Cornish-Bowden, A., and M. L. Cardenas.** 1993. Channelling can affect concentrations of metabolic intermediates at constant net flux: artefact or reality. Eur. J. Biochem. **213:**87–92.
49. **Corre, J., and J. M. Louarn.** 2005. Extent of the activity domain and possible roles of FtsK in the *Escherichia coli* chromosome terminus. Mol. Microbiol. **56:**1539–1548.
50. **Courcelle, J., J. J. Belle, and C. T. Courcelle.** 2004. When replication travels on damaged templates: bumps and blocks in the road. Res. Microbiol. **155:**231–237.
51. **Cremers, A. F., L. Bosch, and J. E. Mellema.** 1981. Characterization of regular polymerization products of elongation factor EFTu from *Escherichia coli* by electron microscopy and image processing. J. Mol. Biol. **153:**477–486.
52. **Crowther, L. J., R. P. Anantha, and M. S. Donnenberg.** 2004. The inner membrane subassembly of the enteropathogenic *Escherichia coli* bundle-forming pilus machine. Mol. Microbiol. **52:**67–79.
53. **Danchin, A., and A. Henaut.** 1997. The map of the cell is in the chromosome. Curr. Opin. Genet. Dev. **7:**852–854.
54. **Daniel, R. A., and J. Errington.** 2003. Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. Cell **113:**767–776.
55. **Dasgupta, S., and A. Lobner-Olesen.** 2004. Host controlled plasmid replication: *Escherichia coli* minichromosomes. Plasmid **52:**151–168.
56. **Davies, K. M., and P. J. Lewis.** 2003. Localization of rRNA synthesis in *Bacillus subtilis*: characterization of loci involved in transcription focus formation. J. Bacteriol. **185:**2346–2353.
57. **de Boer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1989. A division inhibitor and a topological specificity factor coded for by the minicell locus determine the proper placement of the division site in *Escherichia coli*. Cell **56:**641–649.
58. **Defeu Soufo, H. J., and P. L. Graumann.** 2005. *Bacillus subtilis* actin-like protein MreB influences the positioning of the replication machinery and requires membrane proteins MreC/D and other actin-like proteins for proper localization. BMC Cell Biol. **6:**10.
59. **Defeu Soufo, H. J., and P. L. Graumann.** 2004. Dynamic movement of actin-like proteins within bacterial cells. EMBO Rep. **5:**789–794.
60. **Demain, A. L., M. Newcomb, and J. H. Wu.** 2005. Cellulase, clostridia, and ethanol. Microbiol. Mol. Biol. Rev. **69:**124–154.
61. **Den Blaauwen, T., N. Buddelmeijer, M. E. G. Aarsman, C. M. Hameete, and N. Nanninga.** 1999. Timing of FtsZ assembly in *Escherichia coli*. J. Bacteriol. **181:**5167–5175.
62. **Den Blaauwen, T., A. Lindqvist, J. Lowe, and N. Nanninga.** 2001. Distribution of the *Escherichia coli* structural maintenance of chromosomes (SMC)-like protein MukB in the cell. Mol. Microbiol. **42:**1179–1188.
63. **de Pedro, M. A., J. C. Quintela, J.-V. Höltje, and H. Schwartz.** 1997. Murein segregation in *Escherichia coli*. J. Bacteriol. **179:**2823–2834.
64. **Di Lallo, G., M. Fagioli, D. Barionovi, P. Ghelardini, and L. Paolozzi.** 2003. Use of a two-hybrid assay to study the assembly of a complex multicomponent protein machinery: bacterial septosome differentiation. Microbiology **149:**3353–3359.
65. **Dingman, C. W.** 1974. Bidirectional chromosome replication: some topological considerations. J. Theor. Biol. **43:**187–195.
66. **Divakaruni, A. V., R. R. Loo, Y. Xie, J. A. Loo, and J. W. Gober.** 2005. The cell-shape protein MreC interacts with extracytoplasmic proteins including cell wall assembly complexes in *Caulobacter crescentus*. Proc. Natl. Acad. Sci. USA **102:**18602–18607.
67. **Doi, R. H., and A. Kosugi.** 2004. Cellulosomes: plant-cell-wall-degrading enzyme complexes. Nat. Rev. Microbiol. **4:**541–551.
68. **Dowhan, W., E. Mileykovskaya, and M. Bogdanov.** 2004. Diversity and versatility of lipid-protein interactions revealed by molecular genetic approaches. Biochim. Biophys. Acta **1666:**19–39.
69. **Dworkin, J., and R. Losick.** 2002. Does RNA polymerase help drive chromosome segregation in bacteria? Proc. Natl. Acad. Sci. USA **99:**14089–14094.
70. **Dye, N. A., Z. Pincus, J. A. Theriot, L. Shapiro, and Z. Gitai.** 2005. Two independent spiral structures control cell shape in *Caulobacter*. Proc. Natl. Acad. Sci. USA **102:**18608–18613.
71. **Eisenmesser, E. Z., O. Millet, W. Labeikovsky, D. M. Korzhnev, M. Wolf-Watz, D. A. Bosco, J. J. Skalicky, L. E. Kay, and D. Kern.** 2005. Intrinsic dynamics of an enzyme underlies catalysis. Nature **438:**117–121.
72. **Errington, J., R. A. Daniel, and D. J. Scheffers.** 2003. Cytokinesis in bacteria. Microbiol. Mol. Biol Rev. **67:**52–65.
73. **Fekete, R. A., and D. K. Chattoraj.** 2005. A cis-acting sequence involved in chromosome segregation in *Escherichia coli*. Mol. Microbiol. **55:**175–183.
74. **Fennell-Fezzie, R., S. D. Gradia, D. Akey, and J. M. Berger.** 2005. The MukF subunit of *Escherichia coli* condensin: architecture and functional relationship to kleisins. EMBO J. **24:**1921–1930.
75. **Ferguson, P. L., and G. S. Shaw.** 2004. Human S100B protein interacts with the Escherichia coli division protein FtsZ in a calcium-sensitive manner. J. Biol. Chem. **279:**18806–18813.
76. **Figge, R. M., A. V. Divakaruni, and J. W. Gober.** 2004. MreB, the cell shape-determining bacterial actin homologue, coordinates cell wall morphogenesis in *Caulobacter crescentus*. Mol. Microbiol. **51:**1321–1332.

77. **Firshein, W.** 1989. Role of the DNA/membrane complex in prokaryotic DNA replication. Annu. Rev. Microbiol. **43:**89–120.
78. **Fishov, I., and C. Woldringh.** 1999. Visualization of membrane domains in *Escherichia coli*. Mol. Microbiol. **32:**1166–1172.
79. **Formstone, A., and J. Errington.** 2005. A magnesium-dependent *mreB* null mutant: implications for the role of *mreB* in *Bacillus subtilis*. Mol. Microbiol. **55:**1646–1657.
80. **Friedman, N., S. Vardi, M. Ronen, U. Alon, and J. Stavans.** 2005. Precise temporal modulation in the response of the SOS DNA repair network in individual bacteria. PLoS Biol. **3:**1261–1268.
81. **Fulton, A. B., and T. L'Ecuyer.** 1993. Cotranslational assembly of some cytoskeletal proteins: implications and prospects. J. Cell Sci. **105:**867–871.
82. **Furukawa, R., T. M. Jinks, T. Tishgarten, M. Mazzawi, D. R. Morris, and M. Fechheimer.** 2001. Elongation factor 1β is an actin-binding protein. Biochim. Biophys. Acta **1527:**130–140.
83. **Gangola, P., and B. P. Rosen.** 1988. Fura-2 measurements of intracellular [Ca2+] in *Escherichia coli*. Prog. Clin. Biol. Res. **252:**215–220.
84. **Garner, E. C., C. S. Campbell, and R. D. Mullins.** 2004. Dynamic instability in a DNA-segregating prokaryotic actin homolog. Science **306:**1021–1025.
85. **Garvey, N., A. C. St. John, and E. M. Witkin.** 1985. Evidence for RecA protein association with the cell membrane and for changes in the levels of major outer membrane proteins in SOS-induced *Escherichia coli* cells. J. Bacteriol. **163:**870–876.
86. **Geissler, B., and W. Margolin.** 2005. Evidence for functional overlap among multiple bacterial cell division proteins: compensating for the loss of FtsK. Mol. Microbiol. **58:**596–612.
87. **Gitai, Z., N. A. Dye, A. Reisenauer, M. Wachi, and L. Shapiro.** 2005. MreB actin-mediated segregation of a specific region of a bacterial chromosome. Cell **120:**329–341.
88. **Gonzalez, J. M., M. Velez, M. Jimenez, C. Alfonso, P. Schuck, J. Mingorance, M. Vicente, A. P. Minton, and G. Rivas.** 2005. Cooperative behavior of *Escherichia coli* cell-division protein FtsZ assembly involves the preferential cyclization of long single-stranded fibrils. Proc. Natl. Acad. Sci. USA **102:**1895–1900.
89. **Goodwin, B.** 1994. How the leopard changed its spots. Weidenfeld and Nicolson, London, United Kingdom.
90. **Gorringe, D. M., and V. Moses.** 1978. A multienzyme aggregate with glycolytic activity from *Escherichia coli*. Biochem. Soc. Trans. **6:**167–169.
91. **Gowrishankar, J., and R. Harinarayanan.** 2004. Why is transcription coupled to translation in bacteria. Mol. Microbiol. **54:**598–603.
92. **Guzman, E. C., J. L. Caballero, and A. Jimenez-Sanchez.** 2002. Ribonucleoside diphosphate reductase is a component of the replication hyperstructure in *Escherichia coli*. Mol. Microbiol. **43:**487–495.
93. **Hahn, J., B. Maier, B. J. Haijema, M. Sheetz, and D. Dubnau.** 2005. Transformation proteins and DNA uptake localize to the cell poles in *Bacillus subtilis*. Cell **122:**59–71.
94. **Han, S. O., H. Yukawa, M. Inui, and R. H. Doi.** 2005. Effect of carbon source on the cellulosomal subpopulations of *Clostridium cellulovorans*. Microbiology **151:**1491–1497.
95. **Harry, E. J., and P. J. Lewis.** 2003. Early targeting of Min proteins to the cell poles in germinated spores of *Bacillus subtilis*: evidence for division apparatus-independent recruitment of Min proteins to the division site. Mol. Microbiol. **47:**37–48.
96. **Hartwell, L. H., J. J. Hopfield, S. Leibler, and A. W. Murray.** 1999. From molecular to modular cell biology. Nature **402**(Suppl. 6761)**:**C47–C52.
97. **Hegermann, J., R. Herrmann, and F. Mayer.** 2002. Cytoskeletal elements in the bacterium *Mycoplasma pneumoniae*. Naturwissenschaften **89:**453–458.
98. **Helms, M. K., and D. M. Jameson.** 1995. Polymerization of an *Escherichia coli* elongation factor Tu. Arch. Biochem. Biophys. **321:**303–310.
99. **Helmstetter, C. E.** 1991. Timing of synthetic activities in the cell cycle, p. 1594–1605. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. American Society for Microbiology, Washington, DC.
100. **Hon-nami, K., M. P. Coughlan, H. Hon-nami, and L. G. Ljungdahl.** 1986. Separation and characterization of the complexes constituting the cellulolytic enzyme system of *Clostridium thermocellum*. Arch. Microbiol **145:**13–19.
101. **Hu, Y., L. Chen, S. Ha, B. Gross, B. Falcone, D. Walker, M. Mokhtarzadeh, and S. Walker.** 2003. Crystal structure of the MurG:UDP-GlcNAc complex reveals common structural principles of a superfamily of glycosyltransferases. Proc. Natl. Acad. Sci. USA **100:**845–849.
102. **Hu, Z., E. P. Gogol, and J. Lutkenhaus.** 2002. Dynamic assembly of MinD on phospholipid vesicles regulated by ATP and MinE. Proc. Natl. Acad. Sci. USA **99:**6761–6766.
103. **Huang, Y. J., and G. T. Montelione.** 2005. Proteins flex to function. Nature **438:**36–37.
104. **Huitorel, P., and D. Pantaloni.** 1985. Bundling of microtubules by glyceraldehyde-3-phosphate dehydrogenase and its modulation by ATP. Eur. J. Biochem. **150:**265–269.
105. **Ingber, D. E.** 2003. Tensegrity. I. Cell structure and hierarchical systems biology. J. Cell Sci. **116:**1157–1173.
106. **Ip, S. C., M. Bregu, F. X. Barre, and D. J. Sherratt.** 2003. Decatenation of DNA circles by FtsK-dependent Xer site-specific recombination. EMBO J. **22:**6399–6407.
107. **Jeong, H., B. Tombor, R. Albert, Z. N. Oltvai, and A.-L. Barabási.** 2000. The large-scale organization of metabolic networks. Nature **407:**651–654.
108. **Jindal, H. K., and J. K. Vishwanatha.** 1990. Functional identity of a primer recognition protein as phosphoglycerate kinase. J. Biol. Chem. **265:**6540–6543.
109. **Jones, L. J., R. Carballido-Lopez, and J. Errington.** 2001. Control of cell shape in bacteria. Helical, actin-like filaments in *Bacillus subtilis*. Cell **104:**913–922.
110. **Kadoya, R., A. K. Hassan, Y. Kasahara, N. Ogasawara, and S. Moriya.** 2002. Two separate DNA sequences within *oriC* participate in accurate chromosome segregation in *Bacillus subtilis*. Mol. Microbiol. **45:**73–87.
111. **Kang, S., J. S. Han, J. H. Park, K. Skarstad, and D. S. Hwang.** 2003. SeqA protein stimulates the relaxing and decatenating activities of topoisomerase IV. J. Biol. Chem. **278:**48779–48785.
112. **Kaprelyants, A. S., G. V. Mukamolova, H. M. Davey, and D. B. Kell.** 1996. Quantitative analysis of the physiological heterogeneity within starved cultures of *Micrococcus luteus* by flow cytometry and cell sorting. Appl. Environ. Microbiol. **62:**1311–1316.
113. **Karimova, G., N. Dautin, and D. Ladant.** 2005. Interaction network among *Escherichia coli* membrane proteins involved in cell division as revealed by bacterial two-hybrid analysis. J. Bacteriol. **187:**2233–2243.
114. **Karlinsey, J. E., J. Lonner, K. L. Brown, and K. T. Hughes.** 2000. Translation/secretion coupling by type III secretion systems. Cell **102:**487–497.
115. **Kawai, F., M. Shoda, R. Harashima, Y. Sadaie, H. Hara, and K. Matsumoto.** 2004. Cardiolipin domains in *Bacillus subtilis* Marburg membranes. J. Bacteriol. **186:**1475–1483.
116. **Kennell, D., and H. Riezman.** 1977. Transcription and translation frequencies of the *Escherichia coli lac* operon. J. Mol. Biol. **114:**1–21.
117. **Kessler, P. S., and M. Parsons.** 2005. Probing the role of compartmentation of glycolysis in procyclic form Trypanosoma brucei: RNA interference studies of PEX14, hexokinase, and phosphofructokinase. J. Biol. Chem. **280:**9030–9036.
118. **Kidane, D., and P. L. Graumann.** 2005. Dynamic formation of RecA filaments at DNA double strand break repair centers in live cells. J. Cell Biol. **170:**357–366.
119. **Kidane, D., and P. L. Graumann.** 2005. Intracellular protein and DNA dynamics in competent *Bacillus subtilis* cells. Cell **122:**73–84.
120. **Kidane, D., H. Sanchez, J. C. Alonso, and P. L. Graumann.** 2004. Visualization of DNA double-strand break repair in live bacteria reveals dynamic recruitment of *Bacillus subtilis* RecF, RecO and RecN proteins to distinct sites on the nucleoids. Mol. Microbiol. **52:**1627–1639.
121. **Klevecz, R. R., J. Bolen, G. Forrest, and D. B. Murray.** 2004. A genomewide oscillation in transcription gates DNA replication and cell cycle. Proc. Natl. Acad. Sci. USA **101:**1200–1205.
122. **Koppelman, C.-M., T. Den Blaauwen, M. C. Duursma, R. M. A. Heeren, and N. Nanninga.** 2001. *Escherichia coli* minicell membranes are enriched in cardiolipin. J. Bacteriol. **183:**6144–6147.
123. **Kornberg, A., and T. A. Baker.** 1992. DNA replication, 2nd ed. W. H. Freeman, New York, NY.
124. **Kovacs, J., P. Low, A. Pacz, I. Horvath, J. Olah, and J. Ovadi.** 2003. Phosphoenolpyruvate-dependent tubulin-pyruvate kinase interaction at different organizational levels. J. Biol. Chem. **278:**7126–7130.
125. **Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer.** 1994. Biochemistry of homologous recombination in *Escherichia coli*. Microbiol. Rev. **58:**401–465.
126. **Ksenzenko, S. M., and W. S. A. Brusilow.** 1993. Protein-lipid interactions of the proteolipid c subunit of the *Escherichia coli* proton-translocating adenosinetriphosphatase. Arch. Biochem. Biophys. **305:**78–83.
127. **Kumar, J. K., S. Tabor, and C. C. Richardson.** 2004. Proteomic analysis of thioredoxin-targeted proteins in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **101:**3759–3764.
128. **Kurner, J., A. S. Frangakis, and W. Baumeister.** 2005. Cryo-electron tomography reveals the cytoskeletal structure of *Spiroplasma melliferum*. Science **307:**436–438.
129. **Kusano, S., and A. Ishihama.** 1997. Functional interaction of *Escherichia coli* RNA polymerase with inorganic polyphosphate. Genes Cells **2:**433–441.
130. **Kuzminov, A.** 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage λ. Microbiol. Mol. Biol. Rev. **63:**751–813.
131. **Lamed, R., and E. A. Bayer.** 1988. The cellulosome of *Clostridium thermocellum*. Adv. Appl. Microbiol. **33:**1–46.
132. **Lamed, R., and E. A. Bayer.** 1986. Contact and cellulolysis in *Clostridium thermocellum* via extensile surface organelles. Experentia **42:**72–73.
133. **Lara, B., A. I. Rico, S. Petruzzelli, A. Santona, J. Dumas, J. Biton, M. Vicente, J. Mingorance, and O. Massidda.** 2005. Cell division in cocci: localization and properties of the *Streptococcus pneumoniae* FtsA protein. Mol. Microbiol. **55:**699–711.
134. **Lau, I. F., S. R. Filipe, B. Soballe, O. A. Okstad, F. X. Barre, and D. J.**

Sherratt. 2003. Spatial and temporal organization of replicating *Escherichia coli* chromosomes. Mol. Microbiol. **49:**731–743.

135. **Lemke, J. L.** 2000. Opening up closure. Semiotics across scales. Ann. N. Y. Acad. Sci. **901:**100–111.
136. **Lemon, K. P., and A. D. Grossman.** 1998. Localization of bacterial DNA polymerase: evidence for a factory model of replication. Science **282:**1516–1519.
137. **Leonard, A. C., and J. E. Grimwade.** 2005. Building a bacterial orisome: emergence of new regulatory features for replication origin unwinding. Mol. Microbiol. **55:**978–985.
138. **Lesterlin, C., R. Mercier, F. Boccard, F. X. Barre, and F. Cornet.** 13 May 2005. Roles for replichores and macrodomains in segregation of the *Escherichia coli* chromosome. EMBO Rep. [Epub ahead of print.]
139. **Letoffe, S., P. Delepelaire, and C. Wandersman.** 1996. Protein secretion in gram-negative bacteria: assembly of the three components of ABC protein mediated exporters is ordered and promoted by substrate binding. EMBO J. **15:**5804–5811.
140. **Levin, P. A., J. J. Shim, and A. D. Grossman.** 1998. Effect of *minCD* on FtsZ ring position and polar septation in *Bacillus subtilis*. J. Bacteriol. **180:**6048–6051.
141. **Levin-Zaidman, S., D. Frenkiel-Krispin, E. Shimoni, I. Sabanay, S. G. Wolf, and A. Minsky.** 2000. Ordered intracellular RecA-DNA assemblies: a potential site of *in vivo* RecA-mediated activities. Proc. Natl. Acad. Sci. USA **97:**6791–6796.
142. **Levy, O., J. L. Ptacin, P. J. Pease, J. Gore, M. B. Eisen, C. Bustamante, and N. R. Cozzarelli.** 2005. Identification of oligonucleotide sequences that direct the movement of the *Escherichia coli* FtsK translocase. Proc. Natl. Acad. Sci. USA **102:**17618–17623.
143. **Lewis, P. J.** 2004. Bacterial subcellular architecture: recent advances and future prospects. Mol. Microbiol. **54:**1135–1150.
144. **Li, Z., J. L. Kitchen, K. Boeneman, P. Anand, and E. Crooke.** 2005. Restoration of growth to acidic phospholipid-deficient cells by DnaA(L366K) is independent of its capacity for nucleotide binding and exchange and requires DnaA. J. Biol. Chem. **280:**9796–9801.
145. **Lin, C. G., O. Kovalsky, and L. Grossman.** 1997. DNA damage-dependent recruitment of nucleotide excision repair and transcription proteins to *Escherichia coli* inner membranes. Nucleic Acids Res. **25:**3151–3158.
146. **Lin, D. C., and A. D. Grossman.** 1998. Identification and characterization of a bacterial chromosome partitioning site. Cell **92:**675–685.
147. **Lisby, M., U. H. Mortensen, and R. Rothstein.** 2003. Colocalization of multiple DNA double-strand breaks at a single Rad52 repair centre. Nat. Cell Biol. **5:**572–577.
148. **Lisby, M., R. Rothstein, and U. H. Mortensen.** 2001. Rad52 forms DNA repair and recombination centers during S phase. Proc. Natl. Acad. Sci. USA **98:**8276–8282.
149. **Lowe, J., and L. A. Amos.** 1998. Crystal structure of the bacterial cell-division protein FtsZ. Nature **391:**203–206.
150. **Lowe, J., and L. A. Amos.** 1999. Tubulin-like protofilaments in Ca2+-induced FtsZ sheets. EMBO J. **18:**2364–2371.
151. **Lutkenhaus, J., and A. Mukherjee.** 1996. Cell division., p. 1615–1626. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. American Society for Microbiology, Washington, DC.
152. **Lybarger, S. R., and J. R. Maddock.** 2000. Differences in the polar clustering of the high- and low-abundance chemoreceptors of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **97:**8057–8062.
153. **Lynch, A. S., and J. C. Wang.** 1993. Anchoring of DNA to the bacterial cytoplasmic membrane through cotranscriptional synthesis of polypeptides encoding membrane proteins or proteins for export: a mechanism of plasmid hypernegative supercoiling in mutants deficient in DNA topoisomerase I. J. Bacteriol. **175:**1645–1655.
154. **Macnab, R. M.** 2003. How bacteria assemble flagella. Annu. Rev. Microbiol. **57:**77–100.
155. **Maddock, J. R., and L. Shapiro.** 1993. Polar location of the chemoreceptor complex in the *Escherichia coli* cell. Science **259:**1717–1723.
156. **Madkour, M., and F. Mayer.** 2003. Structural organization of the intact bacterial cellulosome as revealed by electron microscopy. Cell Biol. Int. **27:**831–836.
157. **Maniotis, A. J., C. S. Chen, and D. E. Ingber.** 1997. Demonstration of mechanical connections between integins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. Proc. Natl. Acad. Sci. USA **94:** 849–854.
158. **Manning, G. S.** 2006. The contribution of transient counterion imbalances to DNA bending fluctuations. Biophys. J. **90:**3208–3215.
159. **Manning, G. S.** 1969. Limiting laws and counterion condensation in polyelectrolyte solutions. I. Colligative properties. J. Chem. Phys. **51:**924–933.
160. **Marrec-Fairley, M., A. Piette, X. Gallet, R. Brasseur, H. Hara, C. Fraipont, J. M. Ghuysen, and M. Nguyen-Disteche.** 2000. Differential functionalities of amphiphilic peptide segments of the cell-septation penicillin-binding protein 3 of *Escherichia coli*. Mol. Microbiol. **37:**1019–1031.
161. **Mathews, C. K.** 1988. Microcompartmentation of DNA precursors, p. 155–169. *In* D. P. Jones (ed.), Microcompartmentation. CRC Press Inc., Boca Raton, FL.
162. **Matoba, K., M. Yamazoe, K. Mayanagi, K. Morikawa, and S. Hiraga.** 2005. Comparison of MukB homodimer versus MukBEF complex molecular architectures by electron microscopy reveals a higher-order multimerization. Biochem. Biophys. Res. Commun. **333:**694–702.
163. **Mayer, F.** 2003. Cytoskeletons in prokaryotes. Cell Biol. Int. **27:**429–438.
164. **Mayer, F., M. P. Coughlan, Y. Mori, and L. G. Ljungdahl.** 1987. Macromolecular organization of the cellulolytic complex of *Clostridium thermocellum* as revealed by electron microscopy. Appl. Environ. Microbiol. **53:** 2785–2792.
165. **McGlynn, P.** 2004. Links between DNA replication and recombination in prokaryotes. Curr. Opin. Genet. Dev. **14:**107–112.
166. **McGrath, P. T., A. A. Iniesta, K. R. Ryan, L. Shapiro, and H. H. McAdams.** 2006. A dynamically localized protease complex and a polar specificity factor control a cell cycle master regulator. Cell **124:**535–547.
167. **Mendelson, N. H.** 1976. Helical growth of *Bacillus subtilis*: a new model of cell growth. Proc. Natl. Acad. Sci. USA **73:**1740–1744.
168. **Mendes, P., D. B. Kell, and G. R. Welch.** 1995. Metabolic channelling in organized enzyme systems: experiments and models. Adv. Mol. Cell Biol. **11:**1–19.
169. **Mengin-Lecreulx, D., L. Texier, M. Rousseau, and J. van Heijenoort.** 1991. The *murG* gene of *Escherichia coli* codes for the UDP-*N*-acetylglucosamine: *N*-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol *N*-acetylglucosamine transferase involved in the membrane steps of peptidoglycan synthesis. J. Bacteriol. **173:**4625–4636.
170. **Messer, W., U. Belleskes, and H. Lother.** 1985. Effect of dam methylation on the activity of the *E. coli* replication origin, *oriC*. EMBO J. **4:**1327–1332.
171. **Michel, B.** 2005. After 30 years of study, the bacterial SOS response still surprises us. PLoS Biol. **3:**1174–1176.
172. **Michelsen, O., M. J. Teixeira de Mattos, P. R. Jensen, and F. G. Hansen.** 2003. Precise determinations of C and D periods by flow cytometry in *Escherichia coli* K-12 and B/r. Microbiology **149:**1001–1010.
173. **Migocki, M. D., P. J. Lewis, R. G. Wake, and E. J. Harry.** 2004. The midcell replication factory in *Bacillus subtilis* is highly mobile: implications for coordinating chromosome replication with other cell cycle events. Mol. Microbiol. **54:**452–463.
174. **Mileykovskaya, E., and W. Dowhan.** 2005. Role of membrane lipids in bacterial division-site selection. Curr. Opin. Microbiol. **8:**135–142.
175. **Mileykovskaya, E., and W. Dowhan.** 2000. Visualization of phospholipid domains in *Escherichia coli* by using the cardiolipin-specific fluorescent dye 10-*N*-nonyl acridine orange. J. Bacteriol. **182:**1172–1175.
176. **Mileykovskaya, E., I. Fishov, X. Fu, B. D. Corbin, W. Margolin, and W. Dowhan.** 2003. Effects of phospholipid composition on MinD-membrane interactions *in vitro* and *in vivo*. J. Biol. Chem. **278:**22193–22198.
177. **Miller, C. G.** 1996. Protein degradation and proteolytic modification, p. 938–954. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, DC.
178. **Minsky, A.** 2003. Structural aspects of DNA repair: the role of restricted diffusion. Mol. Microbiol. **50:**367–376.
179. **Minsky, A., E. Shimoni, and D. Frenkiel-Krispin.** 2002. Stress, order and survival. Nat. Rev. Mol. Cell Biol. **3:**50–60.
180. **Mishima, O., and H. E. Stanley.** 1998. Decompression-induced melting of ice IV and the liquid-liquid transition in water. Nature **392:**164–168.
181. **Mishima, O., and H. E. Stanley.** 1998. The relationship between liquid, supercooled and glassy water. Nature **396:**329–335.
182. **Mitchell, C. G.** 1996. Identification of a multienzyme complex of the tricarboxylic acid cycle enzymes containing citrate synthase isoenzymes from *Pseudomonas aeruginosa*. Biochem. J. **313:**769–774.
183. **Molina, F., and K. Skarstad.** 2004. Replication fork and SeqA focus distributions in *Escherichia coli* suggest a replication hyperstructure dependent on nucleotide metabolism. Mol. Microbiol. **52:**1597–1612.
184. **Moller-Jensen, J., J. Borch, M. Dam, R. B. Jensen, P. Roepstorff, and K. Gerdes.** 2003. Bacterial mitosis: ParM of plasmid R1 moves plasmid DNA by an actin-like insertional polymerization mechanism. Mol. Cell **12:**1477–1487.
185. **Moller-Jensen, J., R. B. Jensen, J. Lowe, and K. Gerdes.** 2002. Prokaryotic DNA segregation by an actin-like filament. EMBO J. **21:**3119–3127.
186. **Morimatsu, K., and S. C. Kowalczykowski.** 2003. RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange. A universal step of recombinational repair. Mol. Cell **11:**1337–1347.
187. **Mowbray, J., and V. Moses.** 1976. The tentative identification in *Escherichia coli* of a multi-enzyme complex with glycolytic activity. Eur. J. Biochem. **66:**25–36.
188. **Müller-Hill, B.** 1998. The function of auxiliary operators. Mol. Microbiol. **29:**13–18.
189. **Nanninga, N.** 1998. Morphogenesis of *Escherichia coli*. Microbiol. Mol. Biol Rev. **62:**110–129.
190. **Nathan, P., S. L. Gomes, K. Hahnenberger, A. Newton, and L. Shapiro.**

1986. Differential localization of membrane receptor chemotaxis proteins in the *Caulobacter* predivisional cell. J. Mol. Biol. **191:**433–440.
191. **Nielsen, H. J., Y. Li, B. Youngren, F. G. Hansen, and S. Austin.** 2006. Progressive segregation of the Escherichia coli chromosome. Mol. Microbiol. **61:**383–393.
192. **Niki, H., and S. Hiraga.** 1999. Subcellular localization of plasmids containing the *oriC* region of the *Escherichia coli* chromosome, with or without the *sopABC* partitioning system. Mol. Microbiol. **34:**498–503.
193. **Niki, H., Y. Yamaichi, and S. Hiraga.** 2000. Dynamic organization of chromosomal DNA in *Escherichia coli*. Genes Dev. **14:**212–223.
194. **Nishibori, A., J. Kusaka, H. Hara, M. Umeda, and K. Matsumoto.** 2005. Phosphatidylethanolamine domains and localization of phospholipid synthases in *Bacillus subtilis* membranes. J. Bacteriol. **187:**2163–2174.
195. **Noirot-Gros, M. F., E. Dervyn, L. J. Wu, P. Mervelet, J. Errington, S. D. Ehrlich, and P. Noirot.** 2002. An expanded view of bacterial DNA replication. Proc. Natl. Acad. Sci. USA **99:**8342–8347.
196. **Norris, V.** 1992. Phospholipid domains determine the spatial organization of the *Escherichia coli* cell cycle: the membrane tectonics model. J. Theor. Biol. **154:**91–107.
197. **Norris, V., S. Alexandre, Y. Bouligand, D. Cellier, M. Demarty, G. Grehan, G. Gouesbet, J. Guespin, E. Insinna, L. Le Sceller, B. Maheu, C. Monnier, N. Grant, T. Onoda, N. Orange, A. Oshima, L. Picton, H. Polaert, C. Ripoll, M. Thellier, J.-M. Valleton, M.-C. Verdus, J.-C. Vincent, G. White, and P. Wiggins.** 1999. Hypothesis: hyperstructures regulate bacterial structure and the cell cycle. Biochimie **81:**915–920.
198. **Norris, V., P. Amar, G. Bernot, A. Delaune, C. Derue, A. Cabin-Flaman, M. Demarty, Y. Grondin, G. Legent, C. Monnier, H. Pollard, and D. Raine.** 2004. Questions for cell cyclists. J. Biol. Phys. Chem. **4:**124–130.
199. **Norris, V., J. A. Ayala, K. Begg, J.-P. Bouché, P. Bouloc, E. Boye, S. Casaregola, A. J. Cozzone, E. Crooke, R. D'Ari, M. A. de Pedro, W. D. Donachie, R. J. Doyle, G. R. Drapeau, R. Fontana, S. Foster, J. A. Fralick, P. Freestone, R. C. Gayda, M. Goldberg, E. Guzman, J. H. Hageman, C. F. Higgins, M. Hofnung, I. B. Holland, J.-V. Holtje, P. Hughes, M. Inouye, S. Inouye, A. Jaffé, A. Jimenez-Sanchez, D. Joseleau-Petit, W. Keck, F. Kepes, A. Kornberg, P. Kuempel, H. Labischinski, A. Lobner-Olesen, J. Lutkenhaus, P. E. March, M. Matsuhashi, G. McGurk, W. Messer, J. Meury, Y. Milner, K. Modha, K. Nagai, T. Nagata, Y. Nishimura, S. Normark, E. Orr, A. Ottolenghi, L. Paolozzi, P. Poulsen, J. E. Rebollo, E. Z. Ron, J. Rouviere-Yaniv, K. Rudd, G. P. C. Salmond, G. Satta, U. Schwarz, S. Seror, A. Simon, B. G. Spratt, K. Sreekumar, S. Sweeney, I. Toth, R. Utsumi, D. Vinella, M. Wachi, B. M. Wilkins, P. H. Williams, and C. Yanofsky.** 1994. Cell cycle control: prokaryotic solutions to eukaryotic problems? J. Theor. Biol. **168:**227–230.
200. **Norris, V., J. Fralick, and A. Danchin.** 2000. A SeqA hyperstructure and its interactions direct the replication and sequestration of DNA. Mol. Microbiol. **37:**696–702.
201. **Norris, V., P. Gascuel, J. Guespin-Michel, C. Ripoll, and M. H. Saier, Jr.** 1999. Metabolite-induced metabolons: the activation of transporter-enzyme complexes by substrate binding. Mol. Microbiol. **31:**1592–1595.
202. **Norris, V., and G. J. Hyland.** 1997. Do bacteria "sing"? Mol. Microbiol. **24:**879–880.
203. **Norris, V., and M. S. Madsen.** 1995. Autocatalytic gene expression occurs *via* transertion and membrane domain formation and underlies differentiation in bacteria: a model. J. Mol. Biol. **253:**739–748.
204. **Norris, V., C. Woldringh, and E. Mileykovskaya.** 2004. A hypothesis to explain division site selection in *Escherichia coli* by combining nucleoid occlusion and Min. FEBS Lett. **561:**3–10.
205. **Ogino, H., M. Wachi, A. Ishii, N. Iwai, T. Nishida, S. Yamada, K. Nagai, and M. Sugai.** 2004. FtsZ-dependent localization of GroEL protein at possible division sites. Genes Cells **9:**765–771.
206. **Ohsumi, K., M. Yamazoe, and S. Hiraga.** 2001. Different localization of SeqA-bound nascent DNA clusters and MukF-MukE-MukB complex in *Escherichia coli* cells. Mol. Microbiol. **40:**835–845.
207. **Onoda, T., J. Enokizono, H. Kaya, A. Oshima, P. Freestone, and V. Norris.** 2000. Effects of calcium and calcium chelators on growth and morphology of *Escherichia coli* L-form NC-7. J. Bacteriol. **182:**1419–1422.
208. **Onogi, T., H. Niki, M. Yamazoe, and S. Hiraga.** 1999. The assembly and migration of SeqA-Gfp fusion in living cells of *Escherichia coli*. Mol. Microbiol. **31:**1775–1782.
209. **Ovadi, J.** 1988. Old pathway-new concept: control of glycolysis by metabolite-modulated dynamic enzyme associations. Trends Biochem. Sci. **13:**486–490.
210. **Ovadi, J., F. Orosz, and S. Hollan.** 2004. Functional aspects of cellular microcompartmentation in the development of neurodegeneration: mutation induced aberrant protein-protein associations. Mol. Cell. Biochem. *256*-**257:**83–93.
211. **Ovadi, J., and V. Saks.** 2004. On the origin of intracellular compartmentation and organized metabolic systems. Mol. Cell. Biochem. *256*-**257:**5–12.
212. **Pascal, G., C. Medigue, and A. Danchin.** 2005. Universal biases in protein composition of model prokaryotes. Proteins **60:**27–35.
213. **Patel, H. V., K. A. Vyas, X. Li, R. Savtchenko, and S. Roseman.** 2004. Subcellular distribution of enzyme I of the *Escherichia coli* phosphoenolpyruvate:glycose phosphotransferase system depends on growth conditions. Proc. Natl. Acad. Sci. USA **101:**17486–17491.
214. **Pelling, A. E., S. Sehati, E. B. Gralla, J. S. Valentine, and J. K. Gimzewski.** 2004. Local nanomechanical motion of the cell wall of *Saccharomyces cerevisiae*. Science **305:**1147–1150.
215. **Pichoff, S., and J. Luthenhans.** 2002. Unique and overlapping roles for ZipA and FtsA in septal ring assembly in *Escherichis coli*. EMBO J. **21:**685–693.
216. **Pichoff, S., and J. Lutkenhaus.** 2005. Tethering the Z ring to the membrane through a conserved membrane targeting sequence in FtsA. Mol. Microbiol. **55:**1722–1734.
217. **Pollack, G. H.** 2001. Cells, gels and the engines of life: a new, unifying approach to cell function. Ebner and Sons, Seattle, WA.
218. **Popanda, O., G. Fox, and H. W. Thielmann.** 1998. Modulation of DNA polymerases alpha, delta and epsilon by lactate dehydrogenase and 3-phosphoglycerate kinase. Biochim. Biophys. Acta **1397:**102–117.
219. **Raine, D. J., and V. Norris.** 2001. Network structure of metabolic pathways. J. Biol. Phys. Chem. **1:**89–94.
220. **Rajagopalan, S., V. Wachtler, and M. Balasubramanian.** 2003. Cytokinesis in fission yeast: a story of rings, rafts and walls. Trends Genet. **19:**403–408.
221. **Raskin, D. M., and P. A. de Boer.** 1999. Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **96:**4971–4976.
222. **Reski, R.** 2002. Rings and networks: the amazing complexity of FtsZ in chloroplasts. Trends Plant Sci. **7:**103–105.
223. **Reusch, R. N.** 2000. Ion recognition and transport by poly-(R)-3-hydroxybutyrates and inorganic polyphosphates. Adv. Supramol. Chem. **7:**49–98.
224. **Riola, J., E. Guarino, E. C. Guzman, and A. Jimenez-Sanchez.** 2007. Differences in the degree of inhibition of NDP reductase by chemical inactivation and by the thermosensitive mutation nrdA101 in Escherichia coli suggest an effect on chromosome segregation. Cell. Mol. Biol. Lett. **12:**70–81.
225. **Ripoll, C., V. Norris, and M. Thellier.** 2004. Ion condensation and signal transduction. Bioessays **26:**549–557.
226. **Roberts, J., and J.-S. Park.** 2004. Mfd, the bacterial transcription repair coupling factor: translocation, repair and termination. Curr. Opin. Microbiol. **7:**120–125.
227. **Rocha, E., J. Fralick, G. Vediyappan, A. Danchin, and V. Norris.** 2003. A strand-specific model for chromosome segregation in bacteria. Mol. Microbiol. **49:**895–903.
228. **Rocha, E. P., E. Cornet, and B. Michel.** 2005. Comparative and evolutionary analysis of the bacterial homologous recombination systems. PLoS Genet. **1:**0247–0259.
229. **Rokop, M. E., J. M. Auchtung, and A. D. Grossman.** 2004. Control of DNA replication initiation by recruitment of an essential initiation protein to the membrane of *Bacillus subtilis*. Mol. Microbiol. **52:**1757–1767.
230. **Romberg, L., and P. A. Levin.** 2003. Assembly dynamics of the bacterial cell division protein FtsZ: poised at the edge of stability. Annu. Rev. Microbiol. **57:**125–154.
231. **Ronai, Z.** 1993. Glycolytic enzymes as DNA binding proteins. Int. J. Biochem. **25:**1073–1076.
232. **Rosch, J., and M. Caparon.** 2004. A microdomain for protein secretion in Gram-positive bacteria. Science **304:**1513–1515.
233. **Saier, M. H. J.** 2000. Families of transmembrane sugar transport proteins. Mol. Microbiol. **35:**699–710.
234. **Sancar, A.** 1996. DNA excision repair. Annu. Rev. Biochem. **65:**43–81.
235. **Scheffers, D.-J., L. F. Jones, and J. Errington.** 2004. Several distinct localization patterns for penicillin-binding proteins in *Bacillus subtilis*. Mol. Microbiol. **51:**749–764.
236. **Schiffer, G., and J. V. Holtje.** 1999. Cloning and characterization of PBP 1C, a third member of the multimodular class A penicillin-binding proteins of *Escherichia coli*. J. Biol. Chem. **274:**32031–32039.
237. **Sedlak, M.** 2006. Large-scale supramolecular structure in solutions of low molar mass compounds and mixtures of liquids. III. Correlation with molecular properties and interactions. J. Phys. Chem. B **110:**13976–13984.
238. **Shih, Y.-L., T. Le, and L. Rothfield.** 2003. Division site selection in *Escherichia coli* involves dynamic redistribution of Min proteins within coiled structures that extend between the two cell poles. Proc. Natl. Acad. Sci. USA **100:**7865–7870.
239. **Shimmen, T., and E. Yokota.** 2004. Cytoplasmic streaming in plants. Curr. Opin. Cell Biol. **16:**68–72.
240. **Sirover, M. A.** 1999. New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. Biochim. Biophys. Acta **1432:**159–184.
241. **Slovak, P. M., G. H. Wadhams, and J. P. Armitage.** 2005. Localization of MreB in *Rhodobacter sphaeroides* under conditions causing changes in cell shape and membrane structure. J. Bacteriol. **187:**54–64.
242. **Smith, B. T., A. D. Grossman, and G. C. Walker.** 2002. Localization of UvrA and effect of DNA damage on the chromosome of *Bacillus subtilis*. J. Bacteriol. **184:**488–493.
243. **Somers, M., Y. Engelborghs, and J. Baert.** 1990. Analysis of the binding of glyceraldehyde-3-phosphate dehydrogenase to microtubules, the mecha-

nism of bundle formation and the linkage effect. Eur. J. Biochem. **193:**437–444.

244. **Sourjik, V., and H. C. Berg.** 2004. Functional interactions between receptors in bacterial chemotaxis. Nature **428:**437–441.
245. **Srere, P.** 1994. Complexities of metabolic regulation. Trends Biochem. Sci. **19:**519–520.
246. **Srere, P. A.** 1987. Complexes of sequential metabolic enzymes. Annu. Rev. Biochem. **56:**89–124.
247. **Srivastava, D. K., and S. A. Bernhard.** 1986. Metabolite transfer via enzyme-enzyme complexes. Science **234:**1081–1086.
248. **Stein, A., and W. Firshein.** 2000. Probable identification of a membrane-associated repressor of *Bacillus subtilis* DNA replication as the E2 subunit of the pyruvate dehydrogenase complex. J. Bacteriol. **182:**2119–2124.
249. **Stricker, J., P. Maddox, E. D. Salmon, and H. P. Erickson.** 2002. Rapid assembly dynamics of the *Escherichia coli* FtsZ-ring demonstrated by fluorescence recovery after photobleaching. Proc. Natl. Acad. Sci. USA **99:**3171–3175.
250. **Strogatz, S. H., and I. Stewart.** 1993. Coupled oscillators and biological synchronization. Sci. Am. **269:**102–109.
251. **Suefuji, K., R. Valluzzi, and D. RayChaudhuri.** 2002. Dynamic assembly of MinD into filament bundles modulated by ATP, phospholipids, and MinE. Proc. Natl. Acad. Sci. USA **99:**16776–16781.
252. **Tang, J. X., and P. A. Janmey.** 1996. The polyelectrolyte nature of F-actin and the mechanism of actin bundle formation. J. Biol. Chem. **271:**8556–8563.
253. **Teleman, A. A., P. L. Graumann, D. C. Lin, A. D. Grossman, and R. Losick.** 1998. Chromosome arrangement within a bacterium. Curr. Biol. **8:**1102–1109.
254. **Thanbichler, M., and L. Shapiro.** 2006. MipZ, a spatial regulator coordinating chromosome segregation with cell division in Caulobacter. Cell **126:**147–162.
255. **Thanedar, S., and W. Margolin.** 2004. FtsZ exhibits rapid movement and oscillation waves in helix-like patterns in *Escherichia coli*. Curr. Biol. **14:**1167–1173.
256. **Thellier, M., G. Legent, V. Norris, C. Baron, and C. Ripoll.** 2004. Introduction to the concept of "functioning-dependent structures" in living cells. C. R. Biol. **327:**1017–1024.
257. **Thellier, M., C. Ripoll, C. Quintana, F. Sommer, P. Chevallier, and J. Dainty.** 1993. Physical methods to locate metal atoms in biological systems. Methods Enzymol. **227:**535–586.
258. **Tirosh, R.** 2006. Ballistic protons and microwave-induced water solitons in bioenergetic transformations. Int. J. Mol. Sci. **7:**320–345.
259. **Torshin, I.** 1999. Activating oligomerization as intermediate level of signal transduction: analysis of protein-protein contacts and active sites in several glycolytic enzymes. Front. Biosci. **4:**D557–D570.
260. **Travers, A., and G. Muskhelishvili.** 2005. DNA supercoiling—a global transcriptional regulator for enterobacterial growth? Nat. Rev. Microbiol. **3:**157–169.
261. **Trent, J. D., H. K. Kagawa, T. Yaoi, E. Olle, and N. J. Zaluzec.** 1997. Chaperonin filaments: the archaeal cytoskeleton. Proc. Natl. Acad. Sci. USA **94:**5383–5388.
262. **Trushin, M. V.** 2003. The possible role of electromagnetic fields in bacterial communication. J. Microbiol. Immunol. Infect. **36:**153–160.
263. **Tu, B. P., A. Kudlicki, M. Rowicka, and S. L. McKnight.** 2005. Logic of the yeast metabolic cycle: temporal compartmentalization of cellular processes. Science **310:**1152–1158.
264. **Urbanus, M. L., L. Froderberg, D. Drew, P. Bjork, J. W. de Gier, J. Brunner, B. Oudega, and J. Luirink.** 2002. Targeting, insertion, and localization of *Escherichia coli* YidC. J. Biol. Chem. **277:**12718–12723.
265. **Van Den Brink-Van Der Laan, E., J. W. Boots, R. E. Spelbrink, G. M. Kool, E. Breukink, J. A. Killian, and B. De Kruijff.** 2003. Membrane interaction of the glycosyltransferase MurG: a special role for cardiolipin. J. Bacteriol. **185:**3773–3779.
266. **van Den Ent, F., L. A. Amos, and J. Lowe.** 2001. Prokaryotic origin of the actin cytoskeleton. Nature **413:**39–44.
267. **Van Voorst, F., and B. De Kruijff.** 2000. Role of lipids in the translocation of proteins across membranes. Biochem. J. **347:**601–612.
268. **Velot, C., M. B. Mixon, M. Teige, and P. A. Srere.** 1997. Model of a quinary structure between Krebs TCA cycle enzymes: a model for the metabolon. Biochemistry **36:**14271–14276.
269. **Viollier, P. H., M. Thanbichler, P. T. McGrath, L. West, M. Meewan, H. H. McAdams, and L. Shapiro.** 2004. Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication. Proc. Natl. Acad. Sci. USA **101:**9257–9262.
270. **Volker, K. W., C. A. Reinitz, and H. R. Knull.** 1995. Glycolytic enzymes and assembly of microtubule networks. Comp. Biochem. Physiol. **112B:**503–514.
271. **Wada, A., R. Mikkola, C. G. Kurland, and A. Ishihama.** 2000. Growth phase-coupled changes of the ribosome profile in natural isolates and laboratory strains of *Escherichia coli*. J. Bacteriol. **182:**2893–2899.
272. **Wang, L., M. K. Khattar, W. D. Donachie, and J. Lutkenhaus.** 1998. FtsI and FtsW are localized to the septum in *Escherichia coli*. J. Bacteriol. **180:**2810–2816.
273. **Wang, Q., A. Suzuki, S. Mariconda, S. Porwollik, and R. M. Harshey.** 2005. Sensing wetness: a new role for the bacterial flagellum. EMBO J. **24:**2034–2042.
274. **Wang, S. C., L. West, and L. Shapiro.** 2006. The bifunctional FtsK protein mediates chromosome partitioning and cell division in *Caulobacter*. J. Bacteriol. **188:**1497–1508.
275. **Wang, X., C. Possoz, and D. J. Sherratt.** 2005. Dancing around the divisome: asymmetric chromosome segregation in *Escherichia coli*. Genes Dev. **19:**2367–2377.
276. **Watts, D. J., and S. H. Strogatz.** 1998. Collective dynamics of 'small-world' networks. Nature **393:**440–442.
277. **Weiss, D. S.** 2004. Bacterial cell division and the septal ring. Mol. Microbiol. **54:**588–597.
278. **Welch, G. R., and J. S. Easterby.** 1994. Metabolic channeling versus free diffusion: transition-time analysis. Trends Biochem. Sci. **19:**193–196.
279. **Wientjes, F. B., and N. Nanninga.** 1989. Rate and topography of peptidoglycan synthesis during cell division in *Escherichia coli*: concept of a leading edge. J. Bacteriol. **171:**3412–3419.
280. **Wiggins, P. M.** 2002. Enzyme reactions and two-state water. J. Biol. Phys. Chem. **2:**25–37.
281. **Winkel, B. S. J.** 2004. Metabolic channeling in plants. Annu. Rev. Plant Biol. **55:**85–107.
282. **Woldringh, C. L.** 2002. The role of co-transcriptional translation and protein translocation (transertion) in bacterial chromosome segregation. Mol. Microbiol. **45:**17–29.
283. **Woldringh, C. L., P. R. Jensen, and H. V. Westerhoff.** 1995. Structure and partitioning of bacterial DNA determined by a balance of compaction and expansion forces. FEMS Microbiol. Lett. **131:**235–242.
284. **Woldringh, C. L., and N. Nanninga.** 1985. Structure of the nucleoid and cytoplasm in the intact cell, p. 161–197. *In* N. Nanninga (ed.), Molecular cytology of *Escherichia coli*. Academic Press, London, United Kingdom.
285. **Wu, L. J., and J. Errington.** 2004. Coordination of cell division and chromosome segregation by a nucleoid occlusion protein in *Bacillus subtilis*. Cell **117:**915–925.
286. **Wu, L. J., and J. Errington.** 2003. RacA and the Soj-Spo0J system combine to effect polar chromosome segregation in sporulating *Bacillus subtilis*. Mol. Microbiol. **49:**1463–1475.
287. **Yamaichi, Y., and H. Niki.** 2004. *migS*, a cis-acting site that affects bipolar positioning of *oriC* on the *Escherichia coli* chromosome. EMBO J. **23:**221–233.
288. **Yamazoe, M., S. Adachi, S. Kanaya, K. Ohsumi, and S. Hiraga.** 2005. Sequential binding of SeqA protein to nascent DNA segments at replication forks in synchronized cultures of *Escherichia coli*. Mol. Microbiol. **55:**289–298.
289. **Yu, X. C., and W. Margolin.** 1997. Ca^{2+}-mediated GTP-dependent assembly of bacterial cell division protein FtsZ into asters and polymer networks in vitro. EMBO J. **16:**5455–5463.
290. **Zaritsky, A., C. L. Woldringh, R. H. Pritchard, and I. Fishov.** 2000. Surviving bacteria in good shape, p. 347–364. *In* J. Seckbach (ed.), Journey to diverse microbial worlds. Kluwer Academic, Dordrecht, The Netherlands.
291. **Zhang, X., Y. Zhu, and S. Granick.** 2002. Hydrophobicity at a Janus interface. Science **295:**663–666.
292. **Zheng, L., R. G. Roeder, and Y. Luo.** 2003. S phase activation of the histone H2B promoter by OCA-S, a coactivator complex that contains GAPDH as a key component. Cell **114:**255–266.